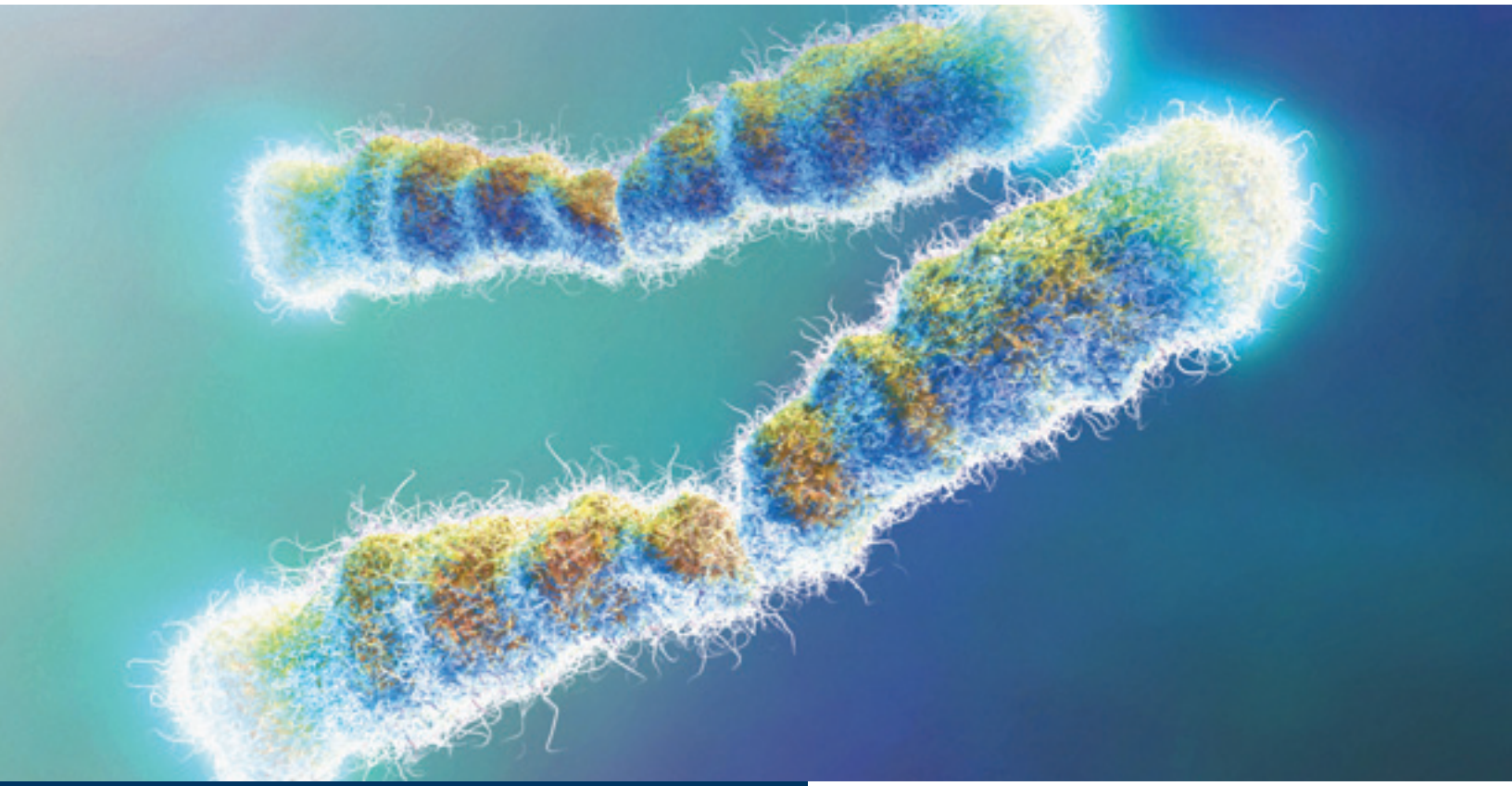
CHAPTER 4

Chromosomes and Sex-Chromosome Inheritance



Chromosomes come in pairs, one from the mother and the other from the father. Human cells have 23 pairs of chromosomes—46 chromosomes altogether. [© Hybrid Medical/Photo Researchers, Inc.]

CHAPTER OUTLINE

LEARNING OBJECTIVES & SCIENCE COMPETENCIES

Understanding how chromosomes and sex-chromosomes are inherited as well as the basic principles of probability and statistical tests of hypotheses discussed in this chapter will allow you to satisfy the following science competencies:

- Predict what products of mitosis or meiosis would result from normal chromosome behavior or chromosome misbehavior.
- Recognize the characteristic pattern of X-linked inheritance, and be able to trace X chromosomes as they pass between the sexes from generation to generation.
- Given a genetic cross, use the binomial distribution to calculate the probability of any particular combination of genotypes or phenotypes among the progeny.
- Be able to formulate a genetic hypothesis, use it to predict expected results of a cross, and compare the expected results with observed results by means of a chi-square test for goodness of fit. Interpret the results of the test according to whether or not the hypothesis should be rejected.

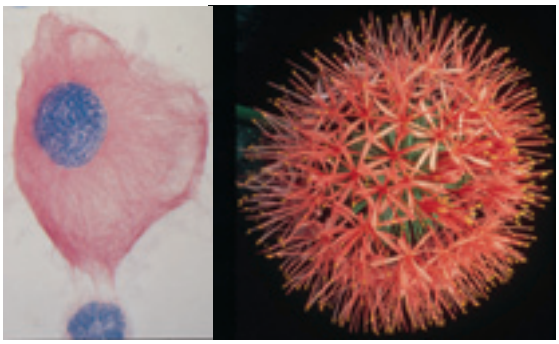
It came as no great revelation that genes are located in chromosomes. The parallel between their properties made this quite obvious:

1. Genes come in pairs; chromosomes come in pairs.
2. Alleles of a gene segregate; homologous chromosomes segregate.
3. Unlinked genes undergo independent assortment; nonhomologous chromosomes undergo independent assortment.

These parallels were first pointed out in 1903, and after that time there was little doubt that chromosomes are the cellular carriers of the genes. But parallels do not constitute scientific proof, nor does widespread agreement among scientists. In this chapter we shall examine some of the experimental evidence that was at the time—and still is—regarded as sufficient to prove the chromosome theory of heredity.

4.1 The Stability of Chromosome Complements

The cell nucleus was first discovered in 1831, but not until the late 1860s was it understood that nuclear division nearly always accompanies cell division. The importance of the nucleus in inheritance was reinforced by the nearly simultaneous discovery that the nuclei of two gametes fuse in the process of fertilization. The next major advance came in the 1880s with the discovery of **chromosomes**, easily visualized in the light microscope with the use of certain dyes. A few years later, chromosomes were found to segregate by an orderly process into the daughter cells formed by cell division as well as into the gametes formed by the division of reproductive cells. Three important regularities were observed about the **chromosome complement** (the complete set of chromosomes) of plants and animals.



MICROTUBULAR cytoskeleton of the African globe amaryllus (*Scadoxus*), which becomes transformed into the spindle in mitosis, as seen through a light microscope. [© Andrew S. Bajer/Bajer Research Projects.]

1. The nucleus of each **somatic cell** (a cell of the body, in contrast to a **germ cell**, or gamete) contains a fixed number of chromosomes typical of the particular species. This number varies tremendously among species, and chromosome number bears little relation to the complexity of the organism (**TABLE 4.1**).
2. The chromosomes in the nuclei of somatic cells are usually present in pairs. For example, the 46 chromosomes of human beings consist of 23

Table 4.1 Somatic (diploid) chromosome numbers of some plant and animal species

Organism	Chromosome number	Organism	Chromosome number
Field horsetail	216	Yeast (*Saccharomyces cerevisiae*)	32
Bracken fern	116	Fruit fly (*Drosophila melanogaster*)	8
Giant sequoia	22	Nematode (*Caenorhabditis elegans*)	11 ♂, 12 ♀
Macaroni wheat	28	House fly	12
Bread wheat	42	Scorpion	4
Fava bean	12	Geometrid moth	224
Garden pea	14	Common toad	22
Mustard cress (*Arabidopsis thaliana*)	10	Chicken	78
Corn (*Zea mays*)	20	Mouse	40
Lily	24	Gibbon	44
Snapdragon	16	Human being	46

pairs (**FIGURE 4.1**). Cells with nuclei that contain two similar sets of chromosomes are called **diploid**. A diploid individual carries two alleleic copies of each gene present in each pair of chromosomes. The chromosomes occur in pairs because one chromosome of each pair derives from the maternal parent and the other from the paternal parent of the organism.

3. The gametes that unite in fertilization to produce the diploid somatic cells have nuclei that contain only one set of chromosomes, consisting of one member of each pair. The gametic nuclei are **haploid**.

FIGURE 4.1 Chromosome complement of a human male. There are 46 chromosomes, present in 23 pairs. At the stage of the division cycle in which these chromosomes were observed, each chromosome consists of two identical halves lying side by side longitudinally. Except for the members of one chromosome pair (the pair that determines sex), the members of all the chromosome pairs are the same color because they contain DNA molecules that were labeled with the same mixture of fluorescent dyes. The colors differ from one pair to the next because the dye mixtures differ in color. In some cases, the long and the short arm have been labeled with a different color. [Courtesy of Michael R. Speicher, Institute of Genome Genetics, Medical University of Graz.]

In multicellular organisms that develop from single cells, the presence of the diploid chromosome number in somatic cells and of the haploid chromosome number in germ cells indicates that there are *two* processes of nuclear division that differ in their outcome. One of these (mitosis) maintains the chromosome number; the other (meiosis) reduces the number by half. These two processes are examined in the following sections.

4.2 Mitosis

Mitosis is a process of nuclear division that ensures that each of two daughter cells receives a diploid complement of chromosomes identical to the diploid complement of the parent cell. Mitosis is usually accompanied by **cytokinesis**, the process in which the cell itself divides to yield two daughter cells. The basic process of mitosis is remarkably uniform in all organisms:

1. Each chromosome is already present as a duplicated structure at the beginning of nuclear division. (The duplication of each chromosome coincides with the replication of the DNA molecule it contains.)
2. Each chromosome divides longitudinally into identical halves that separate from each other.
3. The separated chromosome halves move in opposite directions, and each becomes included in one of the two daughter nuclei that are formed.

In a cell not undergoing mitosis, the chromosomes are invisible with a light microscope. Each consists of an elongated thread too thin to be seen. This stage of the cell cycle is called **interphase**. In preparation for mitosis, the DNA in the chromosomes is replicated during a period of interphase called **S** (FIGURE 4.2), which stands for *synthesis* of DNA. DNA replication is accompanied by chromosome duplication. Before and after S, there are periods, called $\mathbf{G_1}$ and $\mathbf{G_2}$, respectively, in which DNA replication does not take place. The **cell cycle** (the life cycle of a cell) is commonly described in terms of these three interphase periods followed by mitosis, **M**. The order of events is therefore $G_1 \rightarrow S \rightarrow G_2 \rightarrow M$, as shown in Figure 4.2. In this representation, the M period also includes the division of the cytoplasm (cytokinesis) into two approximately equal parts, each containing one daughter nucleus. The length of time required for a complete life cycle varies with cell type; it is 18–24 hours for the majority of cells in higher eukaryotes. The relative duration of the different periods in the cycle also varies considerably with cell type. Mitosis is usually the shortest period, requiring 0.5–2 hours.

The cell cycle is an active, regulated process controlled by mechanisms that are essentially identical in all eukaryotes. These are discussed in detail in Chapter 15. The transitions from G_1 into S and from G_2 into M are called **checkpoints** because the transitions are delayed unless key processes have been completed (Figure 4.2). For example, at the G_1/S checkpoint, either sufficient time must have elapsed since the preceding mitosis (in some cell types) or (in other cell types) the cell must have attained sufficient size for DNA replication to be initiated. Similarly, the G_2/M checkpoint requires that

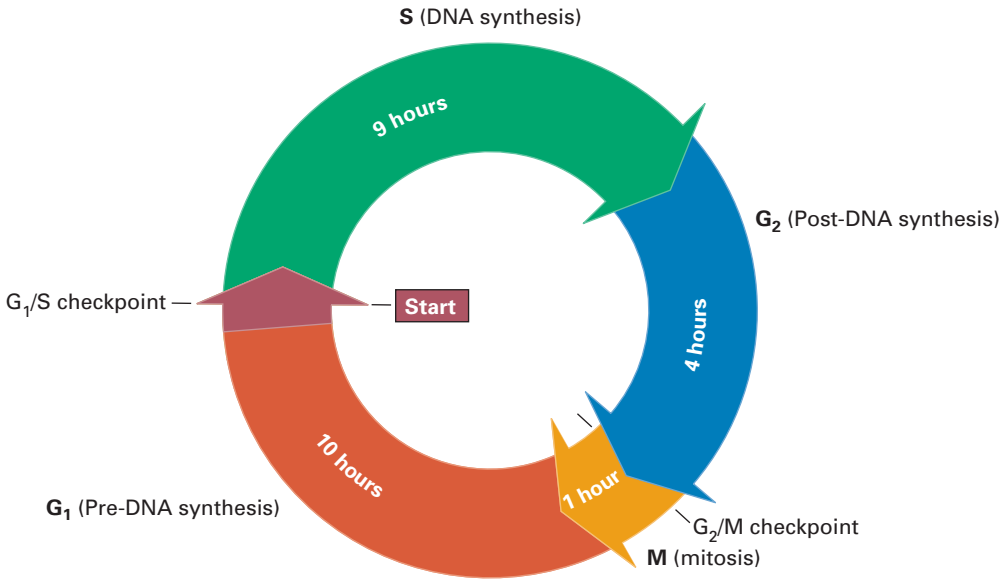
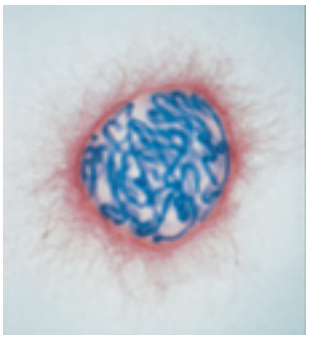


FIGURE 4.2 The cell cycle of a typical mammalian cell growing in tissue culture with a generation time of 24 hours.

DNA replication and repair of any DNA damage be completed for the M phase to commence.

Illustrated in **FIGURE 4.3** are the essential features of chromosome behavior in mitosis. Mitosis is conventionally divided into four stages: **prophase**, **metaphase**, **anaphase**, and **telophase**. (If you have trouble remembering the order, you can jog your memory with "*p*eas *m*ake *a*wful *t*arts.") The stages have the following characteristics:



PROPHASE of *Scadoxus*. [© Andrew S. Bajer/Bajer Research Projects]

1. *Prophase* In interphase, the chromosomes have the form of extended filaments and cannot be seen with a light microscope as discrete bodies. Except for the presence of one or more conspicuous dark bodies (**nucleoli**), the nucleus has a diffuse, granular appearance. The beginning of prophase is marked by the condensation of chromosomes to form visibly distinct threads within the nucleus. Chromatin condensation is brought about by large protein complexes known as *condensins*. At this stage each chromosome is already longitudinally double, consisting of two closely associated subunits called **chromatids**. The longitudinally bipartite nature of each chromosome is readily seen later in prophase. Each pair of chromatids is the product of the duplication of one chromosome in the S period of interphase. The chromatids in a pair are held together at a specific region of the chromosome called the **centromere**. As prophase progresses, the chromosomes become shorter and thicker as a result of intricate coiling (Chapter 7). At the end of prophase, the nucleoli disappear and the nuclear envelope, a membrane surrounding the nucleus, abruptly disintegrates.

METAPHASE of *Scadoxus*. [© Andrew S. Bajer/Bajer Research Projects.]

2. *Metaphase* At the beginning of metaphase, the **mitotic spindle** forms. The spindle is an elongated, football-shaped array of spindle fibers consisting primarily of microtubules formed by polymerization of the protein tubulin. Many other proteins and at least one RNA-protein complex regulate tubulin polymerization and microtubule organization. The ends or *poles* of the spindle, where the microtubules converge, mark the locations of the *centrosomes*, which are the microtubule organizing centers where tubulin polymerization is initiated. Each pair of centrosomes results from the duplication of a single centrosome that takes place in interphase, followed by migration of the daughter centrosomes to opposite sides of the nuclear envelope. (These processes are discussed in more detail in Chapter 15.)

The spindle features three types of microtubules: (1) those that anchor the centrosome to the cell membrane, (2) those that arch between the centrosomes, and (3) those that become attached to the chromosomes. The manner in which these are established exemplifies an important organizing principle of biology that may be called *exploration and stabilization*. As microtubules are formed, new tubulin subunits are added to the growing end of the polymer, but the growing end can also become unstable and initiate a process of depolymerization in which the microtubule shrinks. Several proteins help regulate the balance between polymerization and depolymerization. In spindle formation, microtubules grow out from the spindle poles in essentially random directions (this is the exploration part of the process), but unless something happens to stabilize the growing end, each polymer will ultimately undergo depolymerization and disappear. For the microtubules that become attached to the chromosomes, the event that stabilizes the growing end is contact with a structure technically known as the **kinetochore**, which coincides with the position of the centromere. The process of random exploration and stabilization thereby results in a situation in which only those chromosomal microtubules that make contact with a kinetochore become stabilized and the others depolymerize. Analogous types of stabilization likely also account for the microtubules that attach to the cell membrane and those that arch between the centrosomes.

After the spindle fibers have become attached to the chromosomes, each chromosome is moved to a position near the center of the cell where its kinetochore lies on an imaginary plane approximately equidistant from the spindle poles. This imaginary plane is called the **metaphase plate**. Aligned on the metaphase plate, the chromosomes reach their maximum condensation and are easiest to count and examine for differences in shape and appearance.

Proper chromosome alignment is an important cell cycle control checkpoint at metaphase in both mitosis and meiosis. In a cell in which a chromosome is attached to only one pole of the spindle, the completion of metaphase is delayed. The signal for proper chromosome alignment comes from the kinetochore, and the chemical nature of the signal seems to be the dephosphorylation of certain kinetochore-associated proteins. Through the signaling mechanism, when

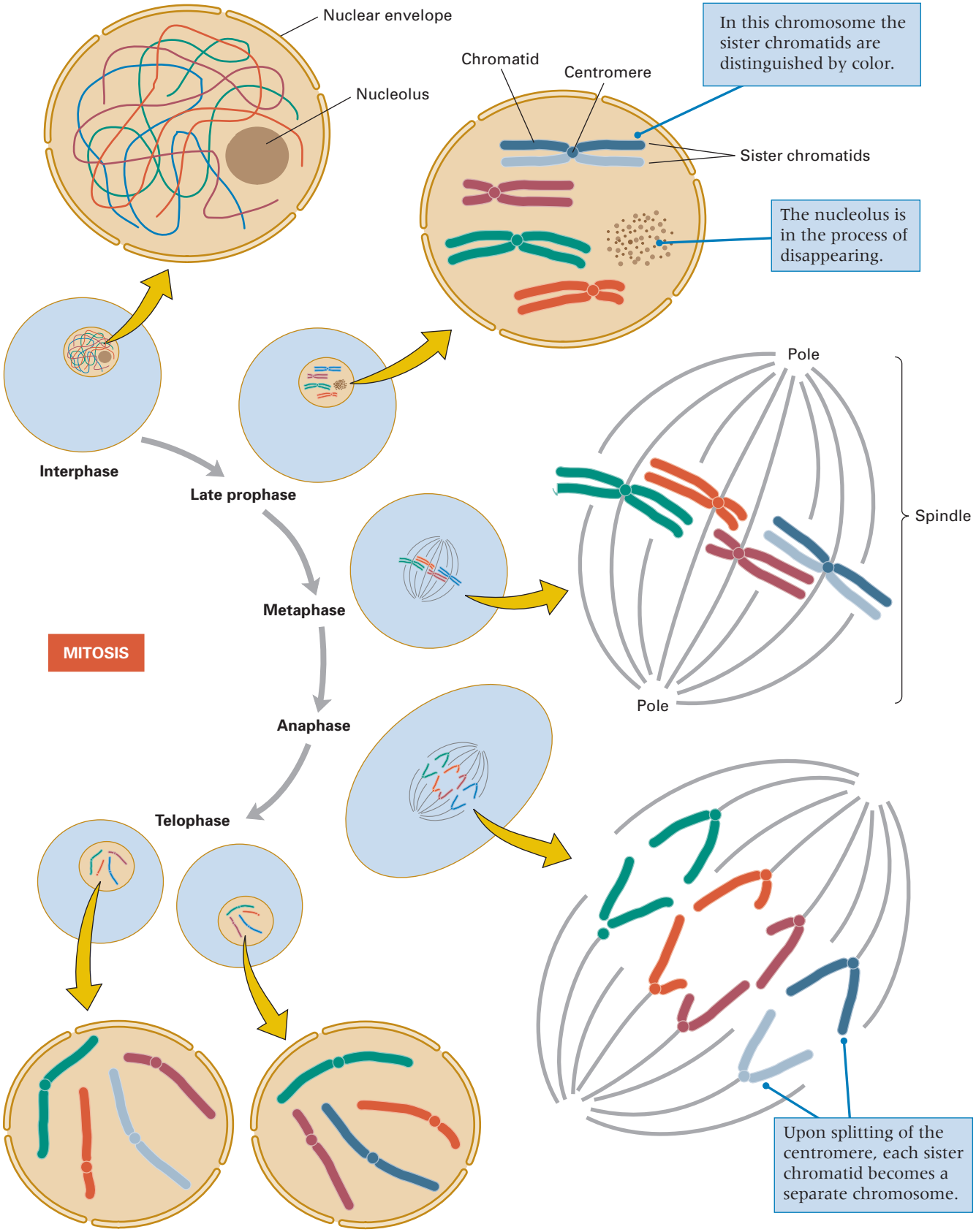
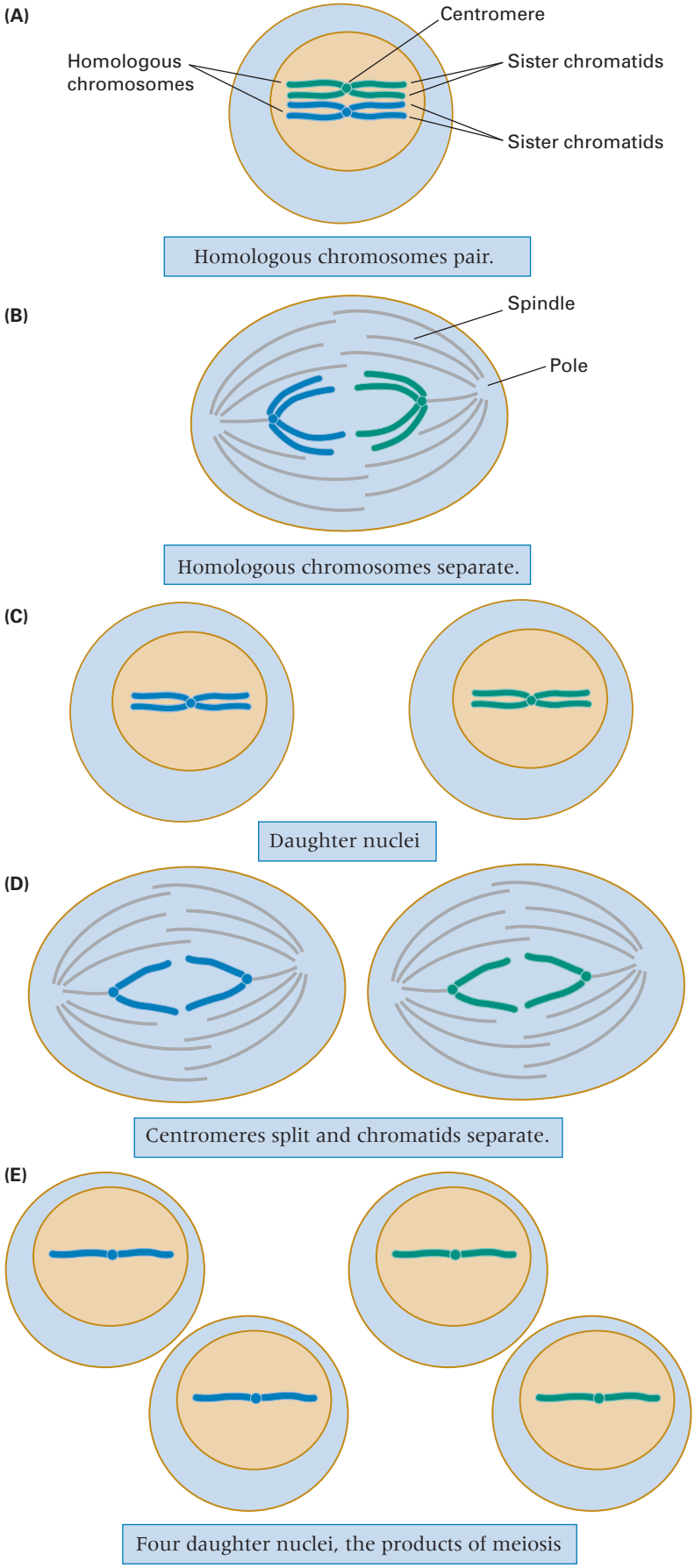
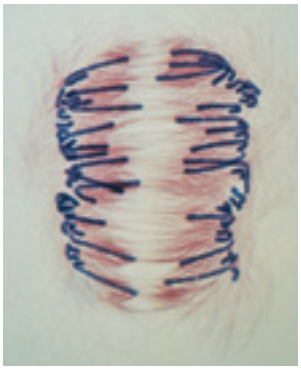


FIGURE 4.3 Chromosome behavior during mitosis in an organism with two pairs of chromosomes (red/rose versus green/blue). At each stage, the smaller inner diagram represents the entire cell, and the larger diagram is an exploded view showing the chromosomes at that stage.



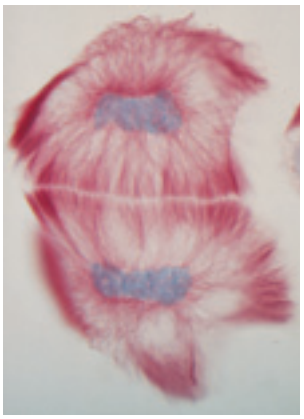
all of the kinetochores are under tension and aligned on the metaphase plate, the metaphase checkpoint is passed and the cell continues the process of division.

3. ***Anaphase*** In anaphase, the proteins holding the chromatids together are dissolved. The centromeres become separate, and the two **sister chromatids** of each chromosome move toward opposite poles of the spindle. Once the centromeres separate, each sister chromatid is regarded as a separate chromosome in its own right. Chromosome movement results in part from progressive shortening of the spindle fibers attached to the centromeres, which pulls the chromosomes in opposite directions toward the poles, and often also from a temporary elongation of the dividing cell in a direction paralleling the spindle. At the completion of anaphase, the chromosomes lie in two groups near opposite poles of the spindle. Each group contains the same number of chromosomes that was present in the original interphase nucleus.



ANAPHASE of *Scadoxus*. [© Andrew S. Bajer/Bajer Research Projects.]

4. ***Telophase*** In telophase, a nuclear envelope forms around each compact group of chromosomes, nucleoli are formed, and the spindle disappears. The chromosomes undergo a reversal of condensation until they are no longer visible as discrete entities. The two daughter nuclei slowly assume a typical interphase appearance as the cytoplasm of the



TELOPHASE of *Scadoxus*. [© Andrew S. Bajer/Bajer Research Projects.]

FIGURE 4.4 Overview of the behavior of a single pair of homologous chromosomes in meiosis. The key differences from mitosis are the pairing of homologous chromosomes (A) and the two successive nuclear divisions (B and D) that reduce the chromosome number by half. For clarity, this diagram does not incorporate crossing over, an interchange of chromosome segments that takes place at the stage depicted in part A.

cell divides into two by means of a gradually deepening furrow around the periphery. (In plants, a new cell wall is synthesized between the daughter cells and separates them.)

4.3 Meiosis

Meiosis is the mode of cell division that results in haploid daughter cells containing only one member of each pair of chromosomes. The process generates genetic diversity because each daughter cell contains a different set of alleles. Meiosis consists of two successive nuclear divisions. An overview of the chromosome behavior is outlined in FIGURE 4.4.

1. Prior to the first nuclear division, the members of each pair of homologous chromosomes become closely associated along their length (Figure 4.4). Each member of the pair is already replicated and consists of two sister chromatids joined at the centromere. The pairing of the homologous chromosomes therefore produces a four-stranded structure.
2. In the first nuclear division, the homologous chromosomes are separated from each other, the members of each pair going to opposite poles of the spindle (Figure 4.4B). Each daughter chromosome consists of two chromatids attached to a common centromere (Figure 4.4C), so both of the two nuclei that are formed contain a haploid set of chromosomes. (Chromosomes are enumerated by counting the number of centromeres, not the number of chromatids.)
3. The second nuclear division loosely resembles a mitotic division, *but there is no DNA replication*. At metaphase, the chromosomes align on the metaphase plate; and at anaphase, the chromatids of each chromosome are separated into opposite daughter nuclei (Figure 4.4D). The net effect of the two divisions in meiosis is the creation of four haploid daughter nuclei, each containing the equivalent of a single sister chromatid from each pair of homologous chromosomes (Figure 4.4E).

Figure 4.4 does not show that the paired homologous chromosomes can exchange genes. The exchanges result in the formation of chromosomes that consist of segments from one homologous chromosome intermixed with segments from the other. In Figure 4.4, the exchanged chromosomes would be depicted as segments of alternating color. The exchange process is one of the critical features of meiosis, and it will be examined in the next section.

In animals, meiosis takes place in specific cells called *meiocytes*, a general term for the primary oocytes and spermatocytes in the gamete-forming tissues (FIGURE 4.5). The *oocytes* form egg

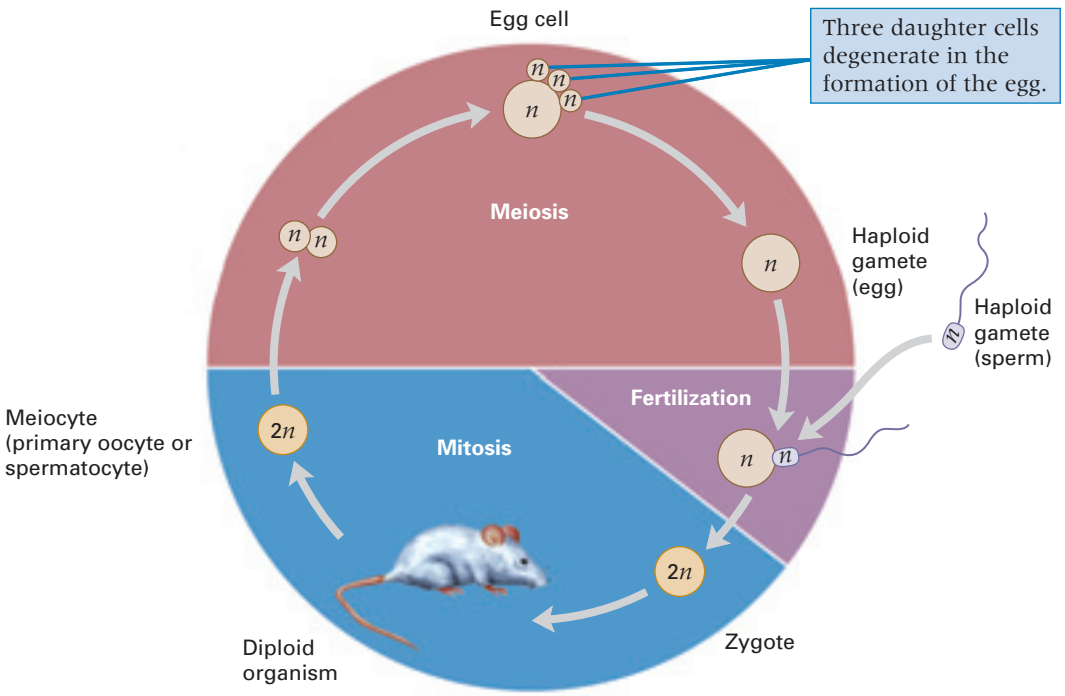


FIGURE 4.5 The life cycle of a typical animal. The number *n* is the number of chromosomes in the haploid chromosome complement. In males, the four products of meiosis develop into functional sperm; in females, only one of the four products develops into an egg.

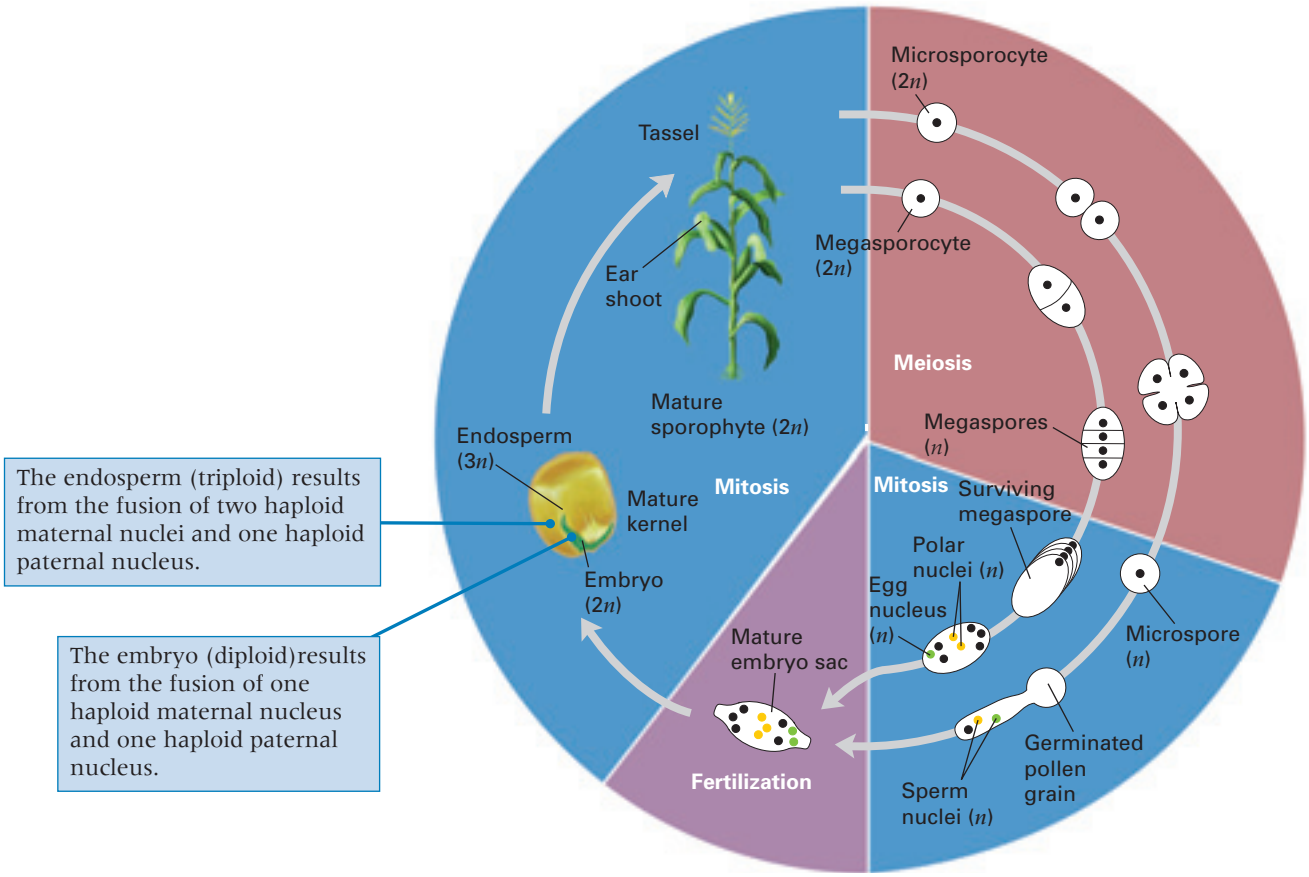
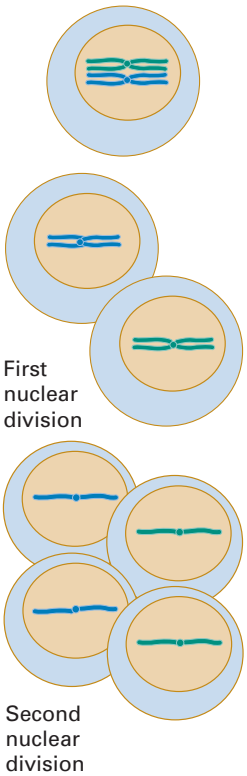


FIGURE 4.6 The life cycle of corn, *Zea mays*. As is typical in higher plants, the diploid spore-producing (sporophyte) generation is conspicuous, whereas the gamete-producing (gametophyte) generation is microscopic. The egg-producing spore is the *megaspore*, and the sperm-producing spore is the *microspore*. Nuclei participating in meiosis and fertilization are shown in yellow and green, respectively.



cells, and the *spermatocytes* form sperm cells. Although the process of meiosis is similar in all sexually reproducing organisms, in the female of both animals and plants, only one of the four products develops into a functional cell while the other three disintegrate. In animals, the products of meiosis form either sperm or eggs. In plants, the situation is slightly more complicated:

1. The products of meiosis typically form *spores*, which undergo one or more mitotic divisions to produce a haploid *gametophyte* organism. The gametophyte produces gametes by mitotic division of a haploid nucleus (**FIGURE 4.6**).
2. Fusion of haploid gametes creates a diploid zygote that develops into the *sporophyte* plant, which undergoes meiosis to produce spores and so restarts the cycle.

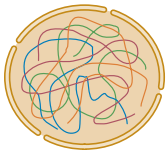
Meiosis is a more complex and considerably longer process than mitosis and usually requires days or even weeks. The entire process of meiosis is illustrated in its cellular context in **FIGURE 4.7**. The essence is that *meiosis consists of two divisions of the nucleus but only one replication of the chromosomes*. The nuclear divisions—called the *first meiotic division* and the *second meiotic division*—can be separated into a sequence of stages similar to those used to describe mitosis. The distinctive events of this important process occur during the first division of the nucleus. These events are described in the following section.

The First Meiotic Division: Reduction

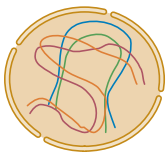
The first meiotic division (meiosis I) is sometimes called the **reductional division** because it divides the chromosome number in half. By analogy with mitosis, the first meiotic division can be split into four stages, which are called **prophase I**, **metaphase I**, **anaphase I**, and **telophase I**. These stages are generally more complex than their counterparts in mitosis. The stages and substages can be visualized with reference to **FIGURES** 4.7 and 4.8.

1. ***Prophase I*** This long stage lasts several days in most higher organisms. It is commonly divided into five substages: *leptotene*, *zygotene*, *pachytene*, *diplotene*, and *diakinesis*. These terms

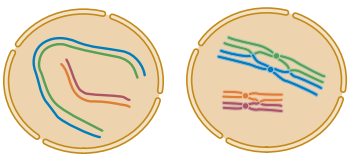
describe the appearance of the chromosomes at each substage.


Leptotene

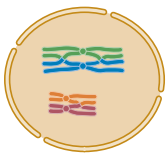
In **leptotene**, which literally means "thin thread," the chromosomes first become visible as long, threadlike structures. The pairs of sister chromatids can be distinguished by electron microscopy. In this initial phase of chromosome condensation, numerous dense granules appear at irregular intervals along their length. These localized contractions, called *chromomeres*, have a characteristic number, size, and position in a given chromosome (Figure 4.8A).


Zygotene

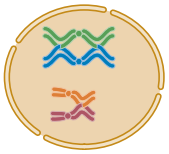
The **zygotene** period is marked by the lateral pairing, or **synapsis**, of homologous chromosomes, beginning at the chromosome tips. (The term *zygotene* means "paired threads.") As the pairing proceeds in zipper-like fashion along the length of the chromosomes, it results in a precise chromomere-by-chromomere association (Figure 4.8B and F). Synapsis is facilitated by the **synaptonemal complex**, a protein structure that helps hold the aligned homologous chromosomes together. Each pair of synapsed homologous chromosomes is referred to as a **bivalent**.


Pachytene

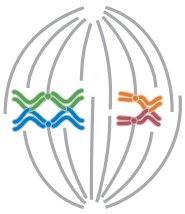
Throughout **pachytene** (Figure 4.8C and D), which literally means "thick thread," the chromosomes continue to shorten and thicken (Figure 4.7). By late pachytene, it can sometimes be seen that each bivalent (that is, each set of paired chromosomes) actually consists of a **tetrad** of four chromatids, but the two sister chromatids of each chromosome are usually juxtaposed very tightly. Genetic exchange by means of **crossing over** takes place during pachytene. In Figure 4.7, the sites of exchange are indicated by the points where chromatids of different colors cross over each other.


Diplotene

At the onset of **diplotene**, the synaptonemal complex breaks down and the synapsed chromosomes begin to separate. Diplotene means "double thread," and the diplotene chromosomes are now clearly double (Figure 4.8E). The pairs of homologous chromosomes remain held together at intervals by cross-connections resulting from crossing over. Each cross-connection is called a **chiasma** (plural, chiasmata) and is formed by a breakage and rejoining between nonsister chromatids. As shown in the chromosome and diagram in FIGURE 4.9, *a chiasma results from physical exchange between chromatids of homologous chromosomes*. In normal meiosis, each bivalent usually has at least one chiasma, and bivalents of long chromosomes often have three or more chiasmata.

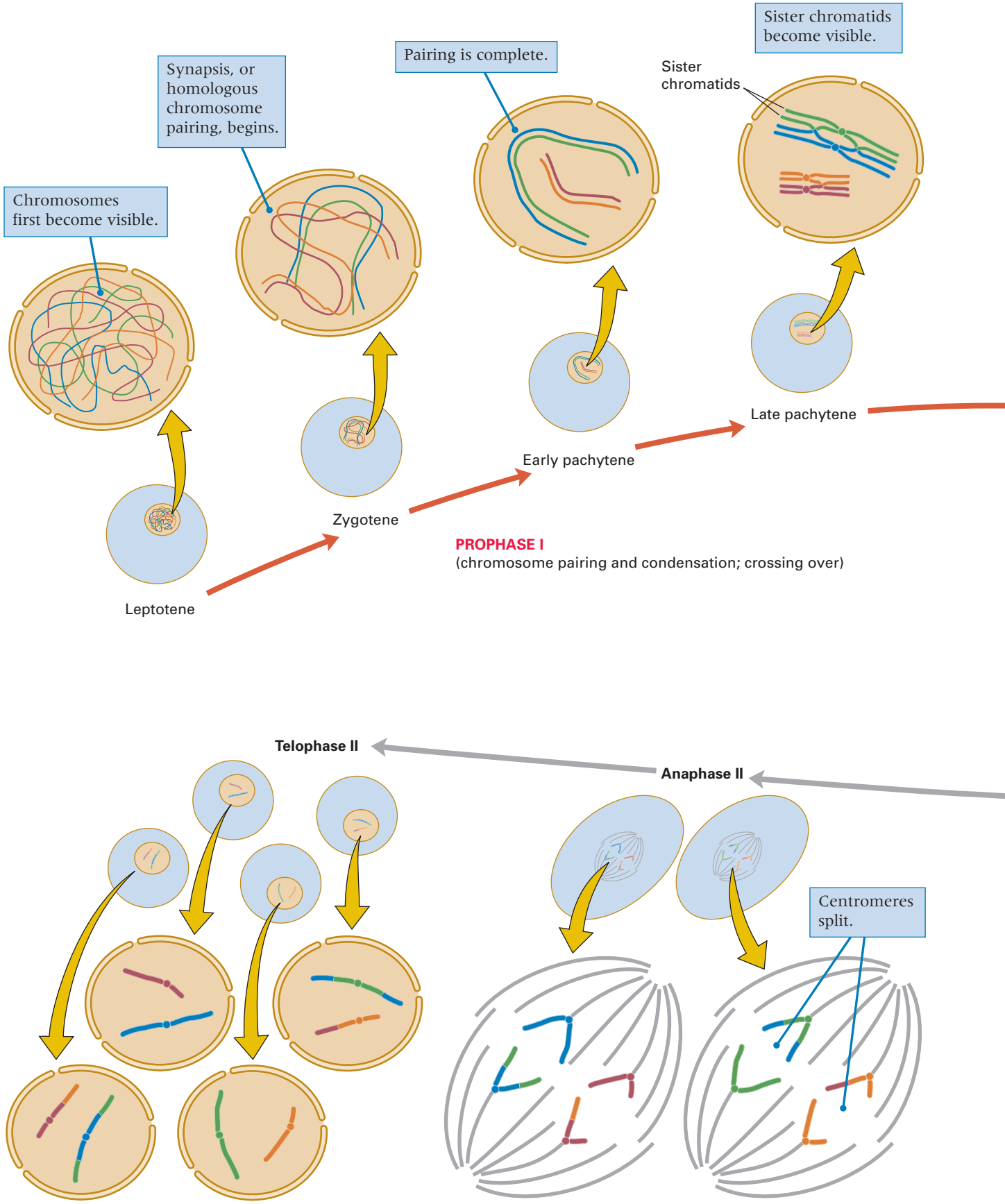

Diakinesis

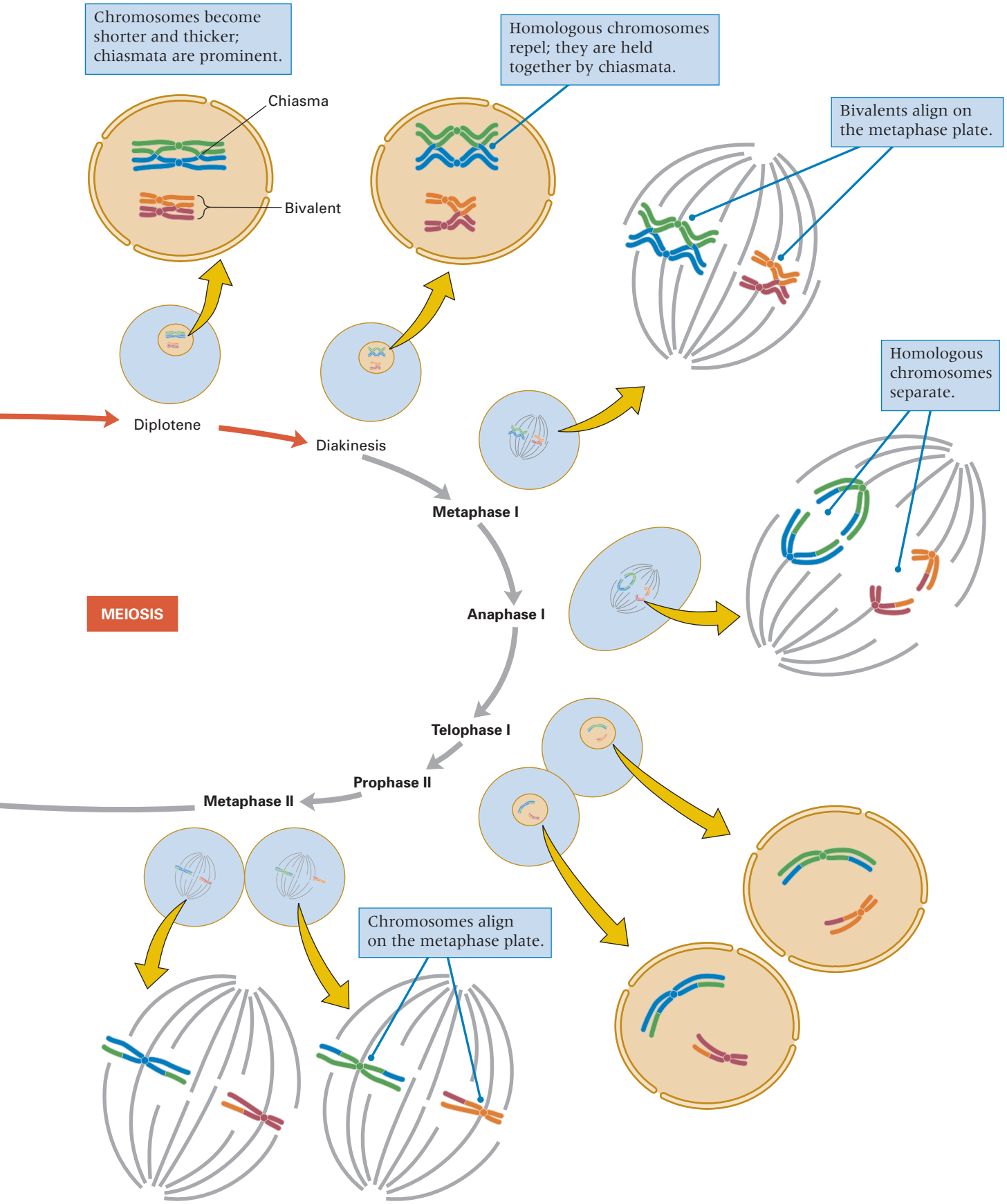
The final period of prophase I is **diakinesis**, in which the homologous chromosomes seem to repel each other and the segments not connected by chiasmata move apart. (Diakinesis means "moving apart.") It is at this substage of prophase I that the chromosomes attain their maximum condensation. The homologous chromosomes in each bivalent remain connected by at least one chiasma, which persists until the first meiotic anaphase. Near the end of diakinesis, the formation of a spindle is initiated, and the nuclear envelope breaks down.


Metaphase I

2. ***Metaphase I*** Each bivalent is maneuvered into a position straddling the metaphase plate with the centromeres of the homologous chromosomes oriented to opposite poles of the spindle (FIGURE 4.10A). The orientation of the centromeres determines which member of each bivalent will subsequently move to each pole, and whether the maternal or the paternal centromere is oriented toward a particular pole is completely a matter of chance. As shown in FIGURE 4.11, the bivalents formed from nonhomologous pairs of chromosomes can be oriented on the metaphase plate in either of two ways. If each of the nonhomologous chromosomes is heterozygous for a pair of alleles, then one type of alignment results in *A B* and *a b* gametes, whereas the other type results in *A b* and *a B* gametes (Figure 4.11). Because the metaphase alignment takes place at random, the two types of alignment—and therefore the four types of gametes—are equally frequent. The ratio of

FIGURE 4.7 Chromosome behavior during meiosis in an organism with two pairs of homologous chromosomes (red/rose and green/blue). At each stage, the small diagram represents the entire cell and the larger diagram is an expanded view of the chromosomes at that stage.





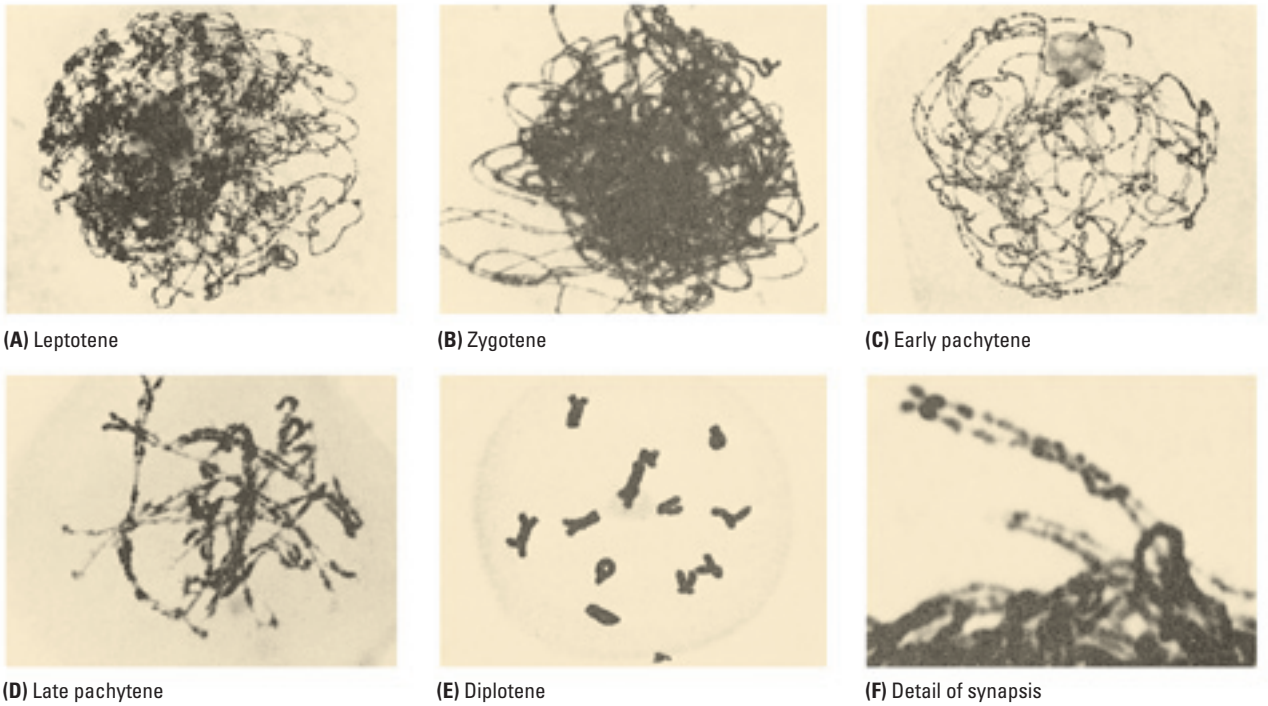


FIGURE 4.8 Substages of prophase I in microsporocytes of the lily (*Lilium longiflorum*). (A) Leptotene: condensation of the chromosomes is initiated and beadlike chromomeres are visible along the length of the chromosomes. (B) Zygotene: pairing (synapsis) of homologous chromosomes occurs (paired and unpaired regions can be seen particularly at the lower left in this photograph). (C) Early pachytene: synapsis is completed and crossing over between homologous chromosomes occurs. (D) Late pachytene: continuation of the shortening and thickening of the chromosomes. (E) Diplotene: mutual repulsion of the paired homologous chromosomes, which remain held together at one or more cross points (chiasmata) along their length. Diakinesis follows (not shown): the chromosomes reach their maximum contraction. (F) Zygotene (at higher magnification in another cell) showing paired homologs and matching of chromomeres during synapsis. [Parts A, B, C, E, and F courtesy of Marta Walters and Santa Barbara Botanic Gardens, Santa Barbara, California. Part D courtesy of Herbert Stern. Used with permission of Ruth Stern.]

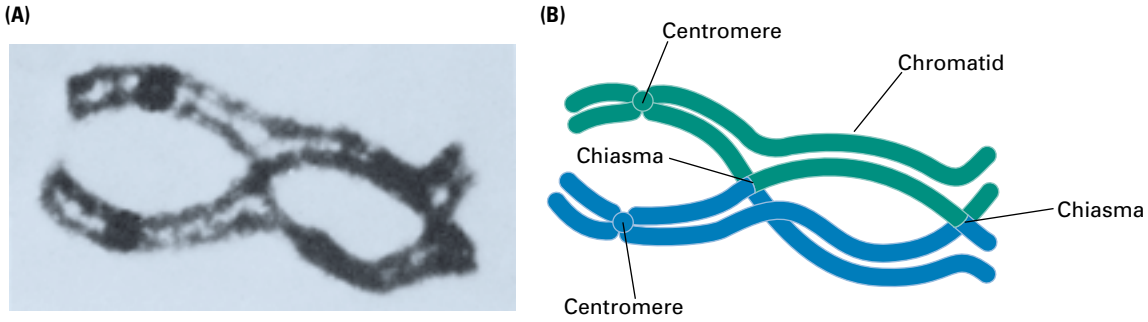


FIGURE 4.9 Light micrograph (A) and interpretive drawing (B) of a bivalent consisting of a pair of homologous chromosomes. This bivalent was photographed at late diplotene in a spermatocyte of the salamander *Oedipina poelzi*. It shows two chiasmata where the chromatids of the homologous chromosomes appear to exchange pairing partners. [Reproduced from *The Mechanics of Inheritance* by Franklin W. Stahl. Copyright © 1964 by Prentice-Hall, Inc. Reprinted by permission of Pearson Education, Inc.]

the four types of gametes is 1 : 1 : 1 : 1, which means that the *A*, *a* and *B*, *b* pairs of alleles undergo independent assortment. That is,

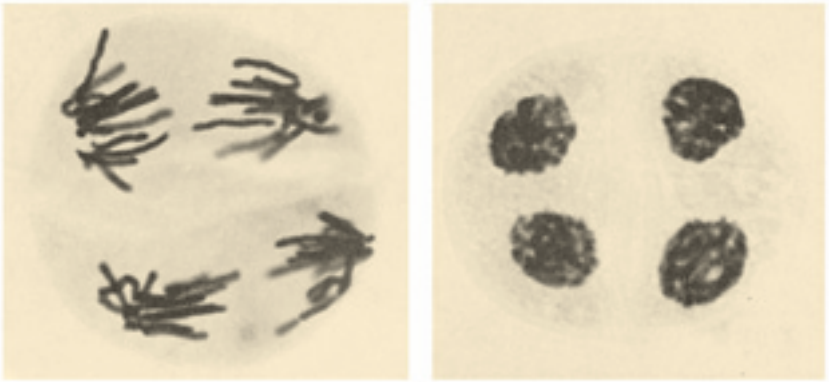
> Genes on different chromosomes undergo independent assortment because nonhomologous chromosomes align at random on the metaphase plate in meiosis I.

3. *Anaphase I* In this stage, homologous chromosomes, each composed of two chromatids joined at an undivided centromere, separate from one another and move to opposite poles of the

(A) Metaphase I

(B) Anaphase I

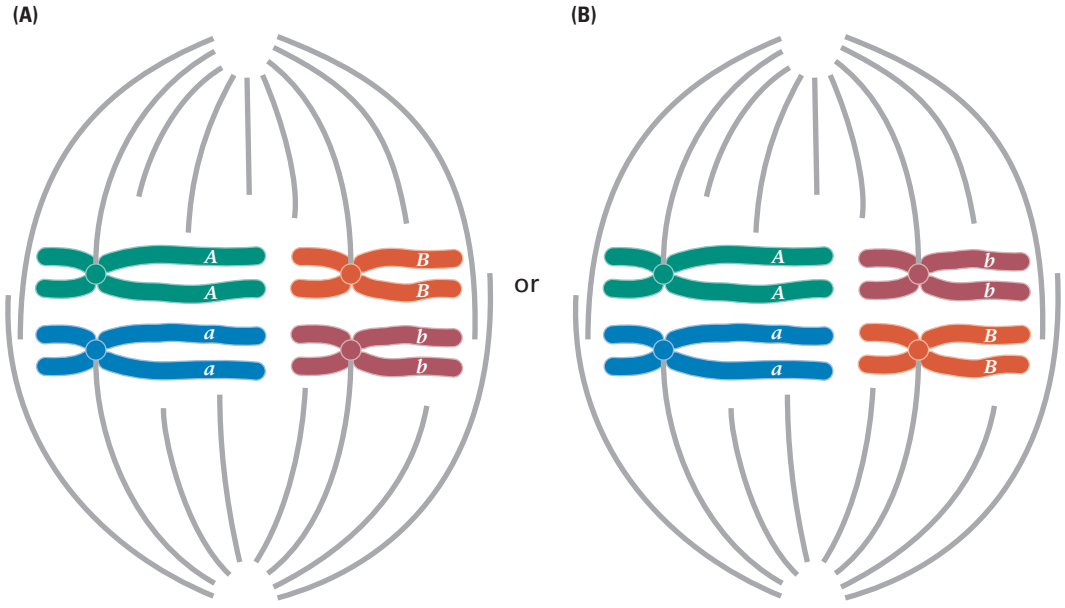
(C) Metaphase II (telophase I and propase II not shown)



(D) Anaphase II

(E) Telophase II

FIGURE 4.10 Later meiotic stages in microsporocytes of the lily *Lilium longiflorum*: (A) metaphase I; (B) anaphase I; (C) metaphase II; (D) anaphase II; (E) telophase II. Cell walls have begun to form in telophase, which will lead to the formation of four pollen grains. [Courtesy of Herbert Stern. Used with permission of Ruth Stern.]



The gametes produced from this alignment are

$A\,B : A\,B : a\,b : a\,b$

The gametes produced from this alignment are

$A\,b : A\,b : a\,B : a\,B$

Because the alignments are equally likely, the overall ratio of gametes is

$$A\,B : A\,b : a\,B : a\,b = 1{:}1{:}1{:}1$$

This ratio is characteristic of independent assortment.

FIGURE 4.11 Independent assortment of genes (A or B) on nonhomologous chromosomes results from random alignment of nonhomologous chromosomes at metaphase I.

connection

Grasshopper, Grasshopper

E. Eleanor Carothers 1913
University of Kansas,
Lawrence, Kansas
The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes

As an undergraduate researcher, Carothers showed that nonhomologous chromosomes undergo independent assortment in meiosis. For this purpose she studied a grasshopper in which one pair of homologous chromosomes had members of unequal length. At the first anaphase of meiosis in males, she could determine by observation whether the longer or the shorter chromosome went in the same direction as the X chromosome. As detailed in this paper, she found 154 of the former and 146 of the latter, a result in very close agreement with the 1 : 1 ratio expected from independent assortment. There is no mention of the Y chromosome because in the grasshopper she studied, the females have the sex chromosome constitution XX, whereas the males have the sex chromosome constitution X. In the males she examined, therefore, the X chromosome did not have a pairing partner. The instrument referred to as a camera lucida was at that time in widespread use for studying chromosomes and other microscopic objects. It is an optical instrument containing a prism or an arrangement of mirrors that, when mounted on a microscope, reflects an image of the microscopic object onto a piece of paper where it can be traced.

The aim of this paper is to describe the behavior of an unequal bivalent in the primary spermatocytes of certain grasshoppers. The distribution of the chromosomes of this bivalent, in relation to the X chromosome, follows the laws of chance; and, therefore, affords direct cytological support of Mendel's laws. This distribution is easily traced on account of a very distinct difference in size of the homologous chromosomes. Thus another link is added to the already long chain of evidence that the chromosomes are distinct morphological individuals continuous from generation to generation, and, as such, are the bearers of the hereditary qualities. . . . This work is based chiefly on *Brachystola magna* [a short-horned grasshopper]. . . . The entire complex of chromosomes can be separated into two groups, one containing six small chromosomes and the other seventeen larger ones. [One of the larger ones is the X chromosome.] Examination shows that this group of six small chromosomes is composed of five of about equal size and one decidedly larger. [One of the small ones is the homolog of the decidedly larger one, making this pair of chromosomes unequal in size.] . . . In early metaphases the chromosomes appear as twelve separate individuals [the bivalents]. Side views show the X chromosome in its characteristic position near one pole. . . . Three hundred cells were drawn under the camera lucida to determine the distribution of the chromosomes in the asymmetrical bivalent in relation to the X chromosome. . . . In the 300 cells drawn, the smaller chromosome went to the same nucleus as the X chromosome 146 times, or in 48.7 percent of the cases; and the larger one, 154 times, or in 51.3 percent of the cases. . . . A consideration of the limited number of chromosomes and the large number of characters in any animal or plant will make it evident that each chromosome must control numerous different characters. . . . Since the rediscovery of Mendel's laws, increased knowledge has been constantly bringing into line facts that at first seemed utterly incompatible with them. There is no cytological explanation of any other form of inheritance. . . . It seems to me probable that all inheritance is, in reality, Mendelian.

Another link is added to the already long chain of evidence that the chromosomes are distinct morphological individuals continuous from generation to generation, and, as such, are the bearers of the hereditary qualities.

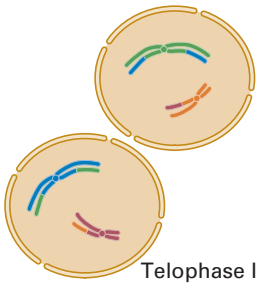
Source: E. E. Carothers, *J. Morphol.* 24 (1913): 487–511.

spindle (Figure 4.10B). Chromosome separation at anaphase is the cellular basis of the segregation of alleles:

> The physical separation of homologous chromosomes in anaphase is the physical basis of Mendel's principle of segregation.

Note, however, that the centromeres of the sister chromatids are tightly stuck together and behave as a single unit. A specific protein acts as a glue holding the sister centromeres together. This protein appears in the centromeres and adjacent chromosome arms during S phase and persists throughout meiosis I. It disappears only at anaphase II, when sister-centromere cohesion is lost and the sister centromeres separate.

4. ***Telophase I*** At the completion of anaphase I, a haploid set of chromosomes consisting of one homolog from each bivalent is located near each pole of the spindle (Figure 4.6). In telophase,

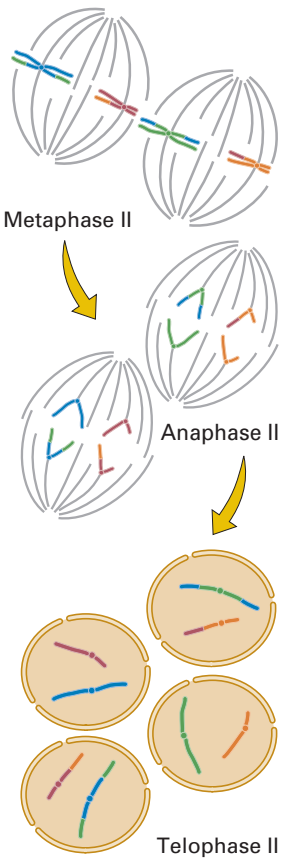

Telophase I

the spindle breaks down, and, depending on the species, either a nuclear envelope briefly forms around each group of chromosomes or the chromosomes enter the second meiotic division after only a limited uncoiling.

The Second Meiotic Division: Equation

The second meiotic division (meiosis II) is sometimes called the **equational division** because the chromosome number remains the same in each cell before and after the second division. In some species, the chromosomes pass directly from telophase I to **prophase II** without loss of condensation; in others, there is a brief pause between the two meiotic divisions and the chromosomes may "decondense" (uncoil) somewhat. *Chromosome replication never takes place between the two divisions*; the chromosomes present at the beginning of the second division are identical to those present at the end of the first.

After a short prophase (prophase II) and the formation of second-division spindles, the centromeres of the chromosomes in each nucleus become aligned on the central plane of the spindle at **metaphase II** (Figure 4.10C). In **anaphase II** the protein holding the sister centromeres together breaks down. As a result, the sister centromeres appear to split longitudinally, and the chromatids of each chromosome move to opposite poles of the spindle (Figure 4.10D). Once the centromeres split at anaphase II, each chromatid is considered to be a separate chromosome.



Telophase II (Figure 4.10E) is marked by a transition to the interphase condition of the chromosomes in the four haploid nuclei, accompanied by division of the cytoplasm. Thus, the second meiotic division superficially resembles a mitotic division. However, there is an important difference:

> The chromatids of a chromosome are usually not genetically identical along their entire length because of crossing over associated with the formation of chiasmata during prophase of the first division.

4.4 Sex-Chromosome Inheritance

The first rigorous experimental proof that genes are parts of chromosomes was obtained in experiments concerned with the pattern of transmission of the **sex chromosomes**, the chromosomes responsible for determination of the separate sexes in some plants and in nearly all animals. We will examine these results in this section.

Chromosomal Determination of Sex

The sex chromosomes are an exception to the rule that all chromosomes of diploid organisms are present in pairs of morphologically similar homologs. As early as 1891, microscopic analysis had shown that one of the chromosomes in males of some insect species does not have a homolog. This unpaired chromosome was called the **X chromosome**, and it was present in all somatic cells of the males but in only half the sperm cells. The biological significance of these observations became clear when females of the same species were shown to have two X chromosomes.

In other species in which the females have two X chromosomes, the male has one X chromosome along with a morphologically unmatched chromosome. The unmatched chromosome is referred to as the **Y chromosome**, and it pairs with the X chromosome during meiosis in males, usually along only part of its length because of a limited region of homology. The difference in chromosomal constitution between males and females is a chromosomal mechanism for determining sex at the time of fertilization. Whereas every egg cell contains an X chromosome, half of the sperm cells contain an X chromosome and the rest contain a Y chromosome. Fertilization of an X-bearing egg by an X-bearing sperm results in an XX zygote, which normally develops into a female; and fertilization by a Y-bearing sperm results in an XY zygote, which normally develops into a male (**FIGURE 4.12**). The result is a criss-cross pattern of inheritance of the X chromosome in which a male receives

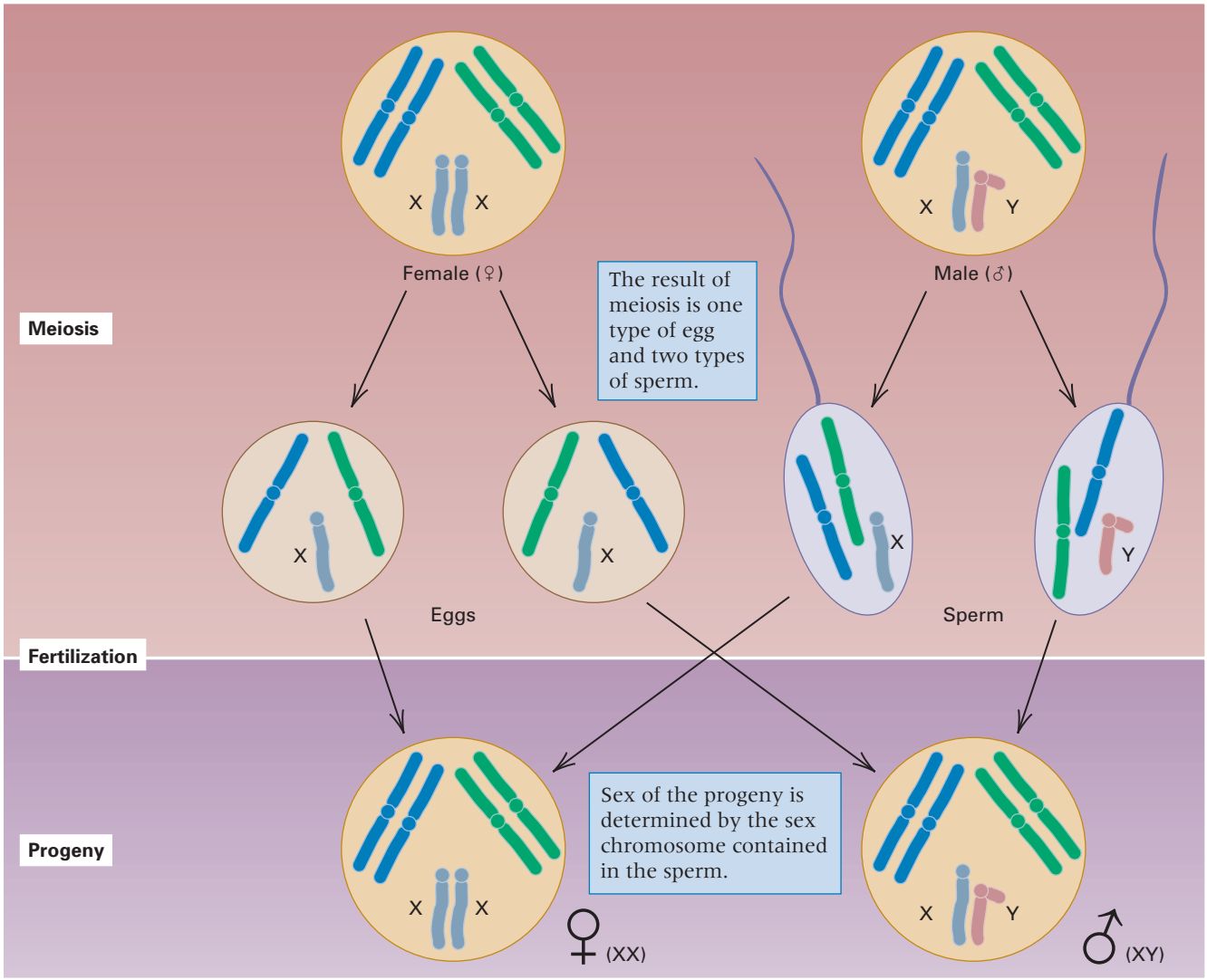


FIGURE 4.12 The chromosomal basis of sex determination in mammals, many insects, and other animals.

his X chromosome from his mother and transmits it only to his daughters.

The XX–XY type of chromosomal sex determination is found in mammals, including human beings, in many insects, and in other animals, as well as in some flowering plants. The female is called the **homogametic** sex because only one type of gamete (X-bearing) is produced, and the male is called the **heterogametic** sex because two different types of gametes (X-bearing and Y-bearing) are produced. When the union of gametes in fertilization is random, a sex ratio at fertilization of 1 : 1 is expected because males produce equal numbers of X-bearing and Y-bearing sperm.

The X and Y chromosomes together constitute the sex chromosomes; this term distinguishes them from other pairs of chromosomes, which are called **autosomes**. Although the sex chromosomes control the developmental switch that determines the earliest stages of female or male development, the developmental process itself requires many genes scattered throughout the genome, including genes on the autosomes. The X chromosome also contains many genes with functions unrelated to sexual differentiation, as we will see in the next section. In most organisms, including human beings, the Y chromosome carries few genes other than those related to male determination.

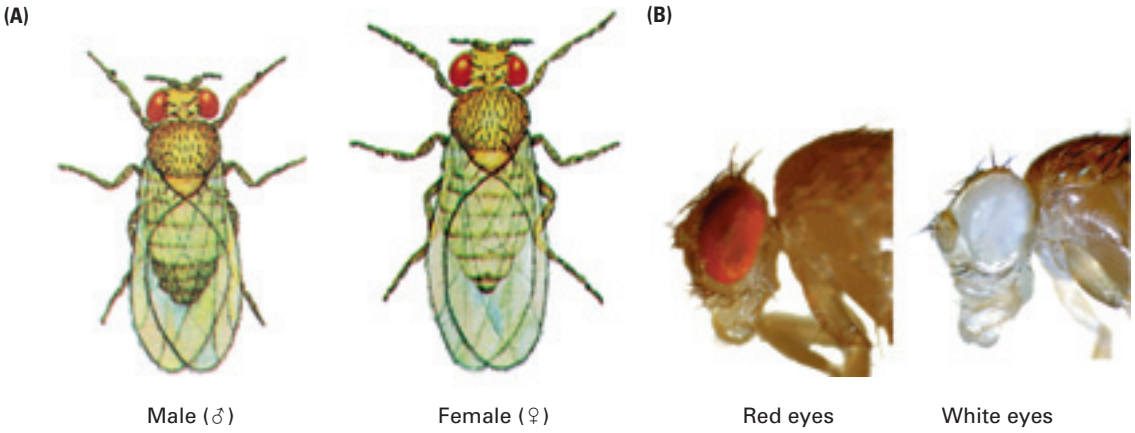


FIGURE 4.13 Drawings (A) of a male and a female fruit fly, *Drosophila melanogaster*. The photographs (B) show the eyes of a wildtype red-eyed male and a mutant white-eyed male. [Illustrations © Carolina Biological Supply Company. Used with permission. Photographs courtesy of E. R. Lozovsky.]

X-Linked Inheritance

The compelling evidence that genes are located in chromosomes came from the study of a *Drosophila* gene for white eyes, which proved to be present in the X chromosome. Recall that in Mendel's crosses, it did not matter which trait was present in the male parent and which in the female parent. Reciprocal crosses gave the same result. One of the earliest exceptions to this rule was found by Thomas Hunt Morgan in 1910 in an early study of a mutant fruit fly that had white eyes. The wildtype eye color is a brick-red combination of red and brown pigments (**FIGURE 4.13**). Although white eyes can result from certain combinations of autosomal genes that eliminate the pigments individually, the white-eye mutation that Morgan studied results in a metabolic block that knocks out both pigments simultaneously. (The gene codes for a transmembrane protein that is necessary to transport the eye pigment precursors into the pigment cells.)

Morgan's study started with a single male with white eyes that appeared in a wildtype laboratory population that had been maintained for many generations. In a mating of this male with wildtype females, all of the F_1 progeny of both sexes had red eyes, which showed that the allele for white eyes is recessive. In the F_2 progeny from the mating of F_1 males with females, Morgan observed 2459 red-eyed females, 1011 red-eyed males, and 782 white-eyed males. It was clear that the white-eyed phenotype was somehow connected with sex, because all of the white-eyed flies were males.

MORGAN'S GROUP partying in the fly room at Columbia University in 1919, to celebrate A. H. Sturtevant's return from military service in World War I. T. H. Morgan is on the far right in the back row, with H. J. Muller beside him. Sturtevant is in the foreground, leaning back in his chair, and C. B. Bridges is in shirtsleeves sitting next to an apelike creature dubbed "Pithecanthropus," a dummy made up for the occasion. [Courtesy of the Archives, California Institute of Technology.]

On the other hand, white eyes were not restricted to males. For example, when red-eyed F_1 females from the cross of wildtype ♀ × white ♂ were backcrossed with their white-eyed fathers, the progeny consisted of both red-eyed and white-eyed females and red-eyed and white-eyed males in approximately equal numbers.

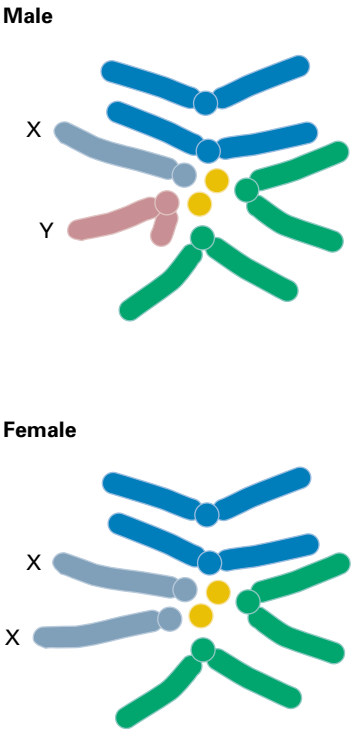


FIGURE 4.14 The diploid chromosome complements of a male and a female *Drosophila melanogaster*. The centromere of the X chromosome is nearly terminal, but that of the Y chromosome divides the chromosome into two unequal arms. The large autosomes (chromosomes 2 and 3, shown in blue and green) are not easily distinguishable in these types of cells. The tiny autosome (chromosome 4, shown in yellow) appears as a dot.

A key observation came from the mating of white-eyed females with wildtype males. All the female progeny had wildtype eyes, but all the male progeny had white eyes. This is the reciprocal of the original cross of wildtype ♀ × white ♂, which had given only wildtype females and wildtype males, so the reciprocal crosses gave different results.

Morgan realized that reciprocal crosses would yield different results if the allele for white eyes were present in the X chromosome. A gene on the X chromosome is said to be **X-linked**. The normal chromosome complement of *Drosophila melanogaster* is shown in FIGURE 4.14. Females have an XX chromosome complement, whereas males are XY. Morgan's hypothesis was that an X chromosome contains either a wildtype w^+ allele or a mutant w allele and that the Y chromosome does not contain a counterpart of the *white* gene. Using the *white* allele present in an X chromosome to represent the entire X chromosome, we can write the genotype of a white-eyed male as wY and that of a wildtype male as w^+Y. Because the w allele is recessive, white-eyed females are of genotype ww and wildtype females are either heterozygous w^+w or homozygous w^+w^+. The implications of this model for reciprocal crosses are shown in FIGURE 4.15. The mating wildtype ♀ × white ♂ is Cross A, and that of white ♀ × wildtype ♂ is Cross B.

The X-linked mode of inheritance does account for the different phenotypic ratios observed in the F_1 and F_2 progeny from the crosses. The characteristics of X-linked inheritance can be summarized as follows:

1. Reciprocal crosses resulting in different phenotypic ratios in the sexes often indicate X-linked inheritance. In the case of white eyes in *Drosophila*, the cross of a red-eyed female with a white-eyed male yields all red-eyed progeny (Figure 4.15, Cross A), whereas the cross of a white-eyed female with a red-eyed male yields red-eyed female progeny and white-eyed male progeny (Figure 4.15, Cross B).
2. Heterozygous females transmit each X-linked allele to approximately half of their daughters and half of their sons; this is illustrated in the F_2 generation of Cross B in Figure 4.15.
3. Males that inherit an X-linked recessive allele exhibit the recessive trait because the Y chromosome does not contain a wildtype counterpart of the gene. Affected males transmit the recessive allele to all of their daughters but none of their sons; this principle is illustrated in the F_1 generation of Cross A in Figure 4.15. Any male that is not affected must carry the wildtype allele in his X chromosome.

The essence of X-linked inheritance is captured in the Punnett square in FIGURE 4.16: A male transmits his X chromosome only to his daughters, whereas a female transmits an X chromosome to offspring of both sexes.

Pedigree Characteristics of Human X-Linked Inheritance

An example of a human trait with an X-linked pattern of inheritance is *hemophilia A*, a severe disorder of blood clotting determined by a recessive allele. Affected persons lack a blood-clotting protein called factor VIII that is needed for normal clotting, and they suffer excessive, often life-threatening bleeding after injury. A famous pedigree of hemophilia starts with Queen

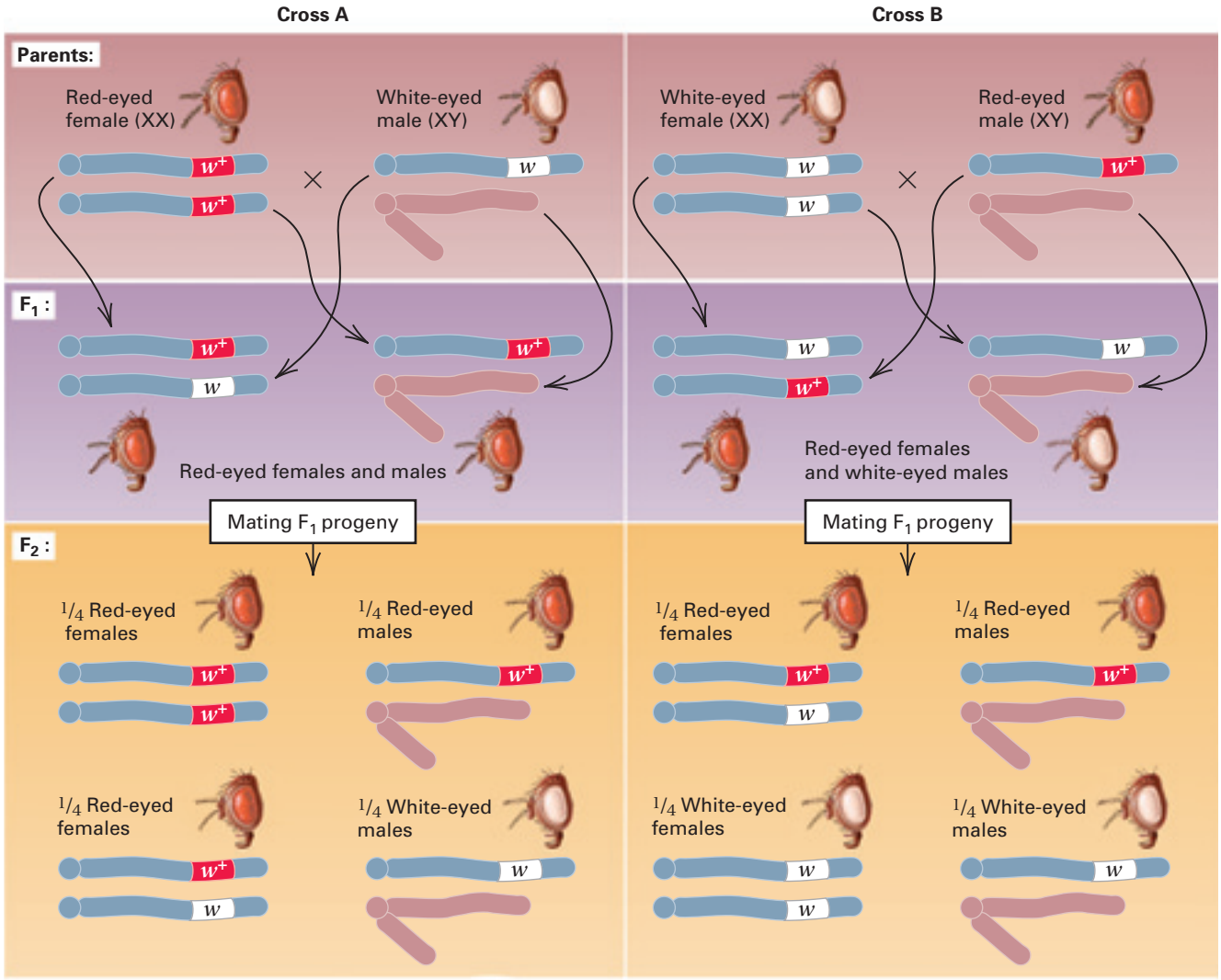


FIGURE 4.15 Chromosomal interpretation of the results obtained in F_1 and F_2 progenies in crosses of *Drosophila*. Cross A is a mating of a wildtype (red-eyed) female with a white-eyed male. Cross B is the reciprocal mating of a white-eyed female with a red-eyed male. In the X chromosome, the wildtype w^+ allele is shown in red, the mutant *w* allele in white. The Y chromosome does not carry either allele of the *w* gene.

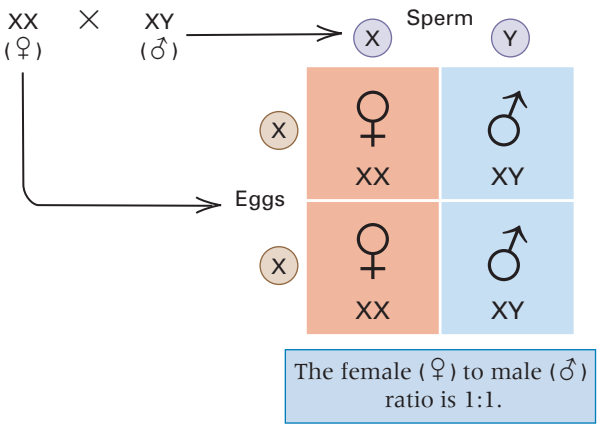


FIGURE 4.16 In chromosomal sex determination, each son gets his X chromosome from his mother and his Y chromosome from his father.

Victoria of England (**FIGURE 4.17**). One of her sons, Leopold, was hemophilic, and two of her daughters were heterozygous carriers of the gene. Two of Victoria's granddaughters were also carriers, and by marriage they introduced the gene into the royal families of Russia and Spain. The heir to the Russian throne of the Romanoffs, Tsarevich Alexis, was afflicted with the condition. He inherited the gene from his mother, the Tsarina Alexandra, one of Victoria's granddaughters. The Tsar, the Tsarina, Alexis, and his four sisters were all executed by the Russian Bolsheviks in the 1918 revolution. Ironically, the present royal family of England is descended from a normal son of Victoria and is free of the disease.

X-linked inheritance in human pedigrees shows several characteristics that distinguish it from other modes of genetic transmission.

1. For any rare trait due to an X-linked recessive allele, the affected individuals

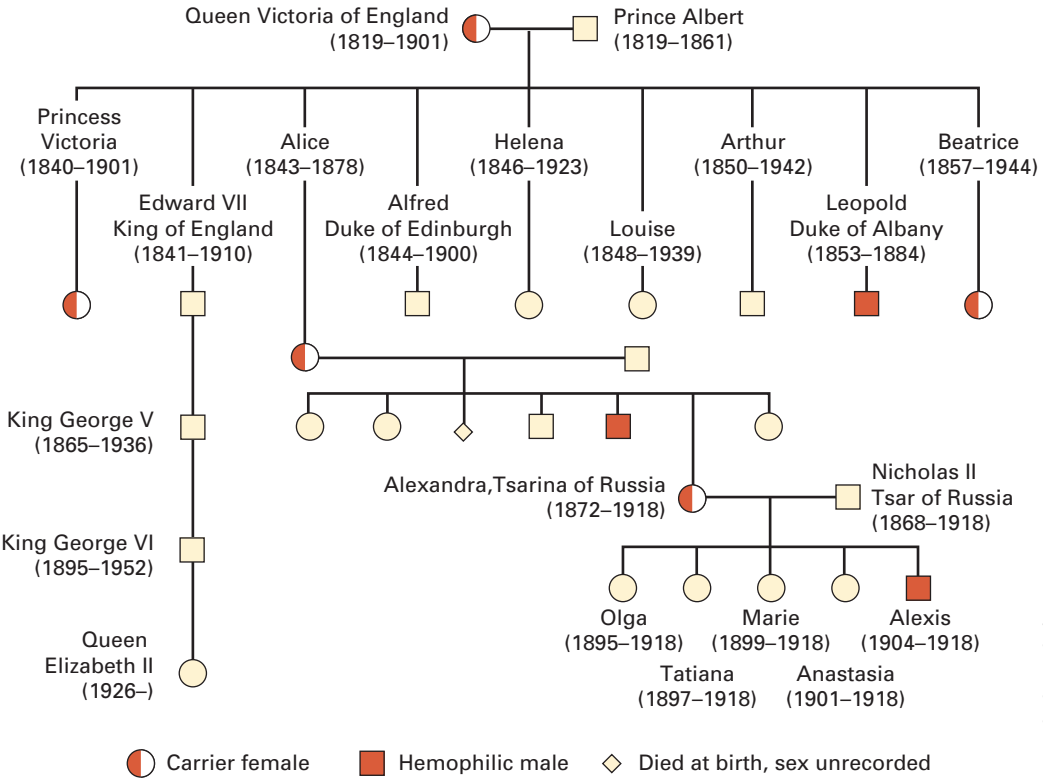


FIGURE 4.17 Genetic transmission of hemophilia A among the descendants of Queen Victoria of England, including her granddaughter, Tsarina Alexandra of Russia, and Alexandra's five children. The photograph is that of Tsar Nicholas II, Tsarina Alexandra, and their children. Tsarevich Alexis was afflicted with hemophilia. [Source: Culver Pictures. Photo courtesy of the Boston Public Library, Print Department.]

are exclusively, or almost exclusively, male. There is an excess of males because females carrying the rare X-linked recessive are almost exclusively heterozygous and so do not express the mutant phenotype.

2. Affected males who reproduce have normal sons. This follows from the fact that a male transmits his X chromosome only to his daughters.
3. A woman whose father was affected has normal sons and affected sons in the ratio 1 : 1. This is true because any daughter of an affected male must be heterozygous for the recessive allele.

Heterogametic Females

In some organisms, the homogametic and heterogametic sexes are reversed; that is, the males are XX and the females are XY. This type of sex determination is found in birds, in some reptiles and fish, and in moths and butterflies. The reversal of XX and XY in the sexes results in an opposite pattern of nonreciprocal inheritance of X-linked genes. To distinguish sex determination in these organisms from the usual XX–XY mechanism, the sex chromosome constitution in the homogametic sex is designated ZZ and that in the heterogametic sex as WZ. Hence in organisms with heterogametic females, the chromosomal constitution of the females is designated WZ and that of the male ZZ.

A specific example of Z-linked inheritance in chickens is shown in FIGURE 4.18. A few breeds of chickens have feathers with alternating

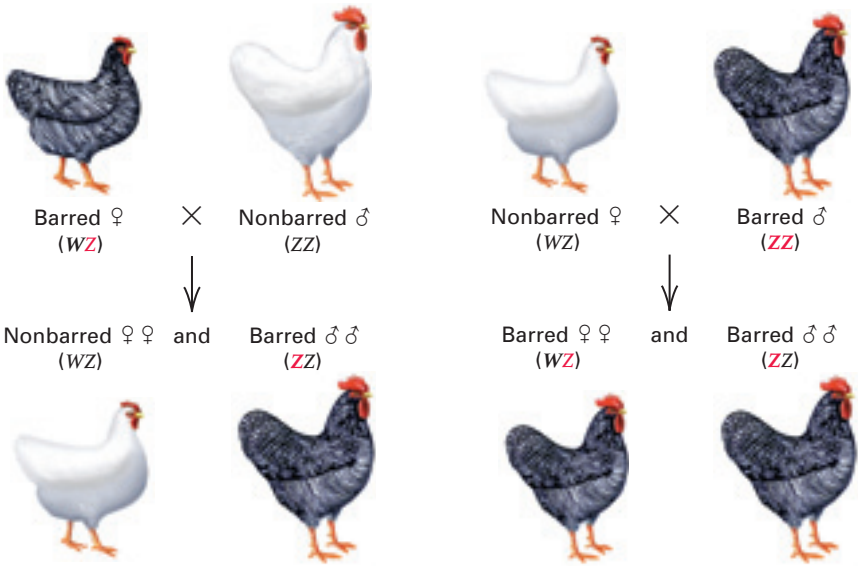


FIGURE 4.18 Barred feathers in chickens, a classic example showing that chromosomal sex determination in birds is the reverse of that in mammals. In birds, females are the heterogametic sex. The Z chromosome carrying the dominant barred mutation is indicated in red.

connection

The White-Eyed Male

Thomas Hunt Morgan 1910
Columbia University,
New York, New York
Sex-Limited Inheritance in Drosophila

Morgan's genetic analysis of the white-eye mutation marks the beginning of Drosophila *genetics. It is in the nature of science that as knowledge increases, the terms used to describe things change also. This paper affords an example, because the term* sex-limited inheritance *is used today to mean something completely different from Morgan's usage. What Morgan was referring to is now called X-linked inheritance or sex-linked inheritance. To avoid confusion, we have taken the liberty of substituting the modern equivalent wherever appropriate. Morgan was also unaware that* Drosophila *males had a Y chromosome. He thought that females were XX and males X, as in grasshoppers (see the Carothers paper). We have also supplied the missing Y chromosome. On the other hand, Morgan's gene symbols have been retained as in the original. He uses* R *for the wildtype allele for red eyes and* W *for the recessive allele for white eyes. This is a curious departure from the convention, already introduced by Mendel, that dominant and recessive alleles should be represented by the same symbol. Today we use* w *for the recessive allele and* w^+ *for the dominant allele.*

In a pedigree culture of *Drosophila* that had been running for nearly a year through a considerable number of generations, a male appeared with white eyes. The normal flies have brilliant red eyes. The white-eyed male, bred to his red-eyed sisters, produced 1,237 red-eyed offspring. . . . The F_1 hybrids, inbred, produced

2,459	red-eyed females
1,011	red-eyed males
782	white-eyed males

No white-eyed females appeared. The new character showed itself to be sex-linked in the sense that it was transmitted only to the grandsons. But that the character is not incompatible with femaleness is shown by the following experiment. The white-eyed male (mutant) was later crossed with some of his daughters (F_1), and produced

No white-eyed females appeared.

129	red-eyed females
132	red-eyed males
88	white-eyed females
86	white-eyed males

The results show that the new character, white eyes, can be carried over to the females by a suitable cross, and is in consequence in this sense not limited to one sex. It will be noted that the four classes of individuals occur in approximately equal numbers (25 percent). . . . The results just described can be accounted for by the following hypothesis. Assume that all of the spermatozoa of the white-eyed male carry the "factor" for white eyes "W"; that half of the spermatozoa carry a sex factor "X," [and] the other half lack it, *i.e.*, the male is heterozygous for sex. [The male is actually XY.] Thus, the symbol for the male is "WXY", and for his two kinds of spermatozoa WX—Y. Assume that all of the eggs of the red-eyed female carry the red-eyed "factor" R; and that all of the eggs (after meiosis) carry one X each, the symbol for the red-eyed female will be therefore RRXX and that for her eggs will be RX. . . . The hypothesis just utilized to explain these results first obtained can be tested in several ways. [There follow four types of crosses, each yielding the expected result.] . . . In order to obtain these results it is necessary to assume that, when the two classes of spermatozoa are formed in the RXY male, R and X go together. . . . The fact is that this R and X are combined and have never existed apart.

Source: T. H. Morgan, *Science* 32 (1910): 120–122.

transverse bands of light and dark color, resulting in a phenotype known as barred. In other breeds the feathers are uniformly colored and nonbarred. Reciprocal crosses between true-breeding barred and true-breeding nonbarred breeds give the results shown, which indicate that the gene that determines barring must be dominant and must be located in the Z chromosome.

Nondisjunction as Proof of the Chromosome Theory of Heredity

The parallel between the inheritance of the *Drosophila white* mutation and the genetic transmission of the X chromosome supported the chromosome theory of heredity that genes are parts of chromosomes. Other experiments with *Drosophila* provided the definitive proof.

One of Morgan's students, Calvin Bridges, discovered rare exceptions to the expected pattern of inheritance in crosses with several X-linked genes. For example, when white-eyed *Drosophila* females were mated with red-eyed males, most of the progeny consisted of the expected red-eyed females and white-eyed males. However, about 1 in every 2000 F_1 flies was an exception, either a white-eyed female or a red-eyed male. Bridges showed that these rare exceptional offspring resulted from occasional failure of the two X chromosomes in the mother to separate from each other during meiosis—a

phenomenon called **nondisjunction**. The consequence of nondisjunction of the X chromosome is the formation of some eggs with two X chromosomes and others with none. Four classes of zygotes are expected from the fertilization of these abnormal eggs (**FIGURE 4.19**). Animals with no X chromosome are not detected because embryos that lack an X are not viable; likewise, most progeny with three X chromosomes die early in development. Microscopic examination of the chromosomes of the exceptional progeny from the cross white ♀ × wild-type ♂ showed that the exceptional white-eyed females had two X chromosomes *plus* a Y chromosome, and the exceptional red-eyed males had a single X but were *lacking* a Y. The latter, with a sex-chromosome constitution denoted XO, were sterile males.

These and related experiments demonstrated conclusively the validity of the chromosome theory of heredity.

Chromosome theory of heredity: Genes are physically located within chromosomes.

Bridges's evidence for the chromosome theory was that exceptional behavior on the part of chromosomes is precisely paralleled by exceptional inheritance of their genes. This proof of the chromosome theory ranks among the most important and elegant experiments in genetics.

Sex Determination in *Drosophila*

In the XX–XY mechanism of sex determination, the Y chromosome is associated with the male. In some organisms, including humans, this association occurs because the presence of the

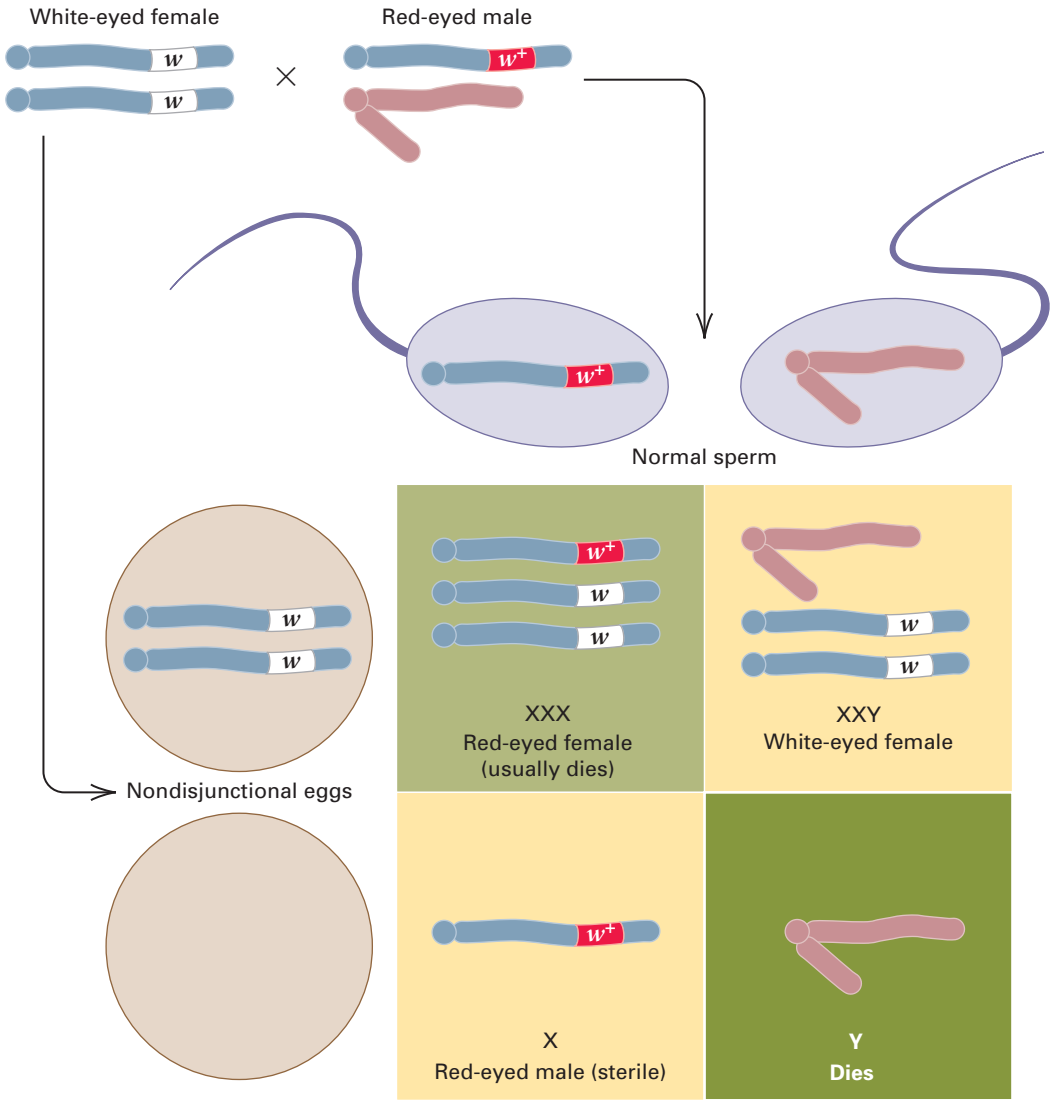


FIGURE 4.19 Results of nondisjunction of the X chromosomes in the first meiotic division in a female *Drosophila*.

Y chromosome triggers events in embryonic development that result in the male sexual characteristics (Chapter 8). *Drosophila* is unusual among organisms with an XX–XY type of sex determination because the Y chromosome, although associated with maleness, is not male determining. This is demonstrated by the finding, shown in **FIGURE 4.20**, that XXY embryos in *Drosophila* develop into morphologically normal, fertile females, whereas XO embryos develop into morphologically normal, but sterile, males. (The O is written in the formula XO to emphasize that a sex chromosome is missing.) The sterility of XO males shows that the Y chromosome, though not necessary for male development, is essential for male fertility; in fact, the *Drosophila* Y chromosome contains six genes required for the formation of normal sperm.

The genetic determination of sex in *Drosophila* depends on an X-linked gene known as Sex-lethal (*Sxl*) because some mutant alleles are lethal when present in males. The *Sxl* gene is active in normal females and inactive in normal males. The product of *Sxl* is the Sex-lethal protein SXL, which is an RNA-binding protein that binds with the RNA transcripts of several genes and causes female-specific RNA processing. In the absence of SXL, these transcripts undergo male-specific processing. Hence the targets of SXL are genes whose protein products are normally made only in females. One of the targets of SXL is the *Sxl* gene itself, so a small burst of SXL activity early in female development leads to self-sustaining production of SXL because of feedback in the processing of more *Sxl* transcripts.

What makes for the early burst of SXL is the amount of product produced by a small number of X-linked genes known as *X-linked signal elements* or XSE (Figure 4.20). If the amount of XSE is high, the early burst occurs and the organism becomes female; whereas if the amount of XSE is low, the early burst does not occur and the organism becomes male (Figure 4.20). For almost 100 years geneticists believed that sex was determined by the ratio of the number of X chromosomes (X) relative to the number of sets of autosomes (A). The correlation is perfect. In normal females (XXAA) the ratio equals 1, in normal males (XAA) it equals 1/2, and in rare individuals of composition XXAAA the ratio is 2/3 and the organism develops as an intersex. Furthermore, rare XA haploids initially develop as female, although they eventually die, which again supports the idea that an embryo with a ratio of X : A of 1/2 develops as male, an embryo with an X : A ratio of 1 develops as a female, and an embryo with an X : A ratio between 1/2 and 1 develops as an intersex.

While the correlation with X : A is perfect, however, recent data show that the hypothesis is wrong. Sex in *Drosophila* is based on *Sxl* reacting to the amount of XSE, which in normal flies is determined by the number of X chromosomes. The autosomes do play a role but not in sex determination. Their role is in determining when in early development the commitment to sexual differentiation takes place. In normal XXAA or XAA embryos with two sets of autosomes, commitment takes place after 15 cycles of division. In XA haploid embryos with one set of autosomes, commitment takes place one cell cycle later when the amount of XSE is sufficient to induce *Sxl*. In XXAAA embryos, some cells commit in cycle 13 and have too little XSE to induce *Sxe*, whereas other cells commit in cycle 14 and have enough XSE to induce *Sxl*. The result is an

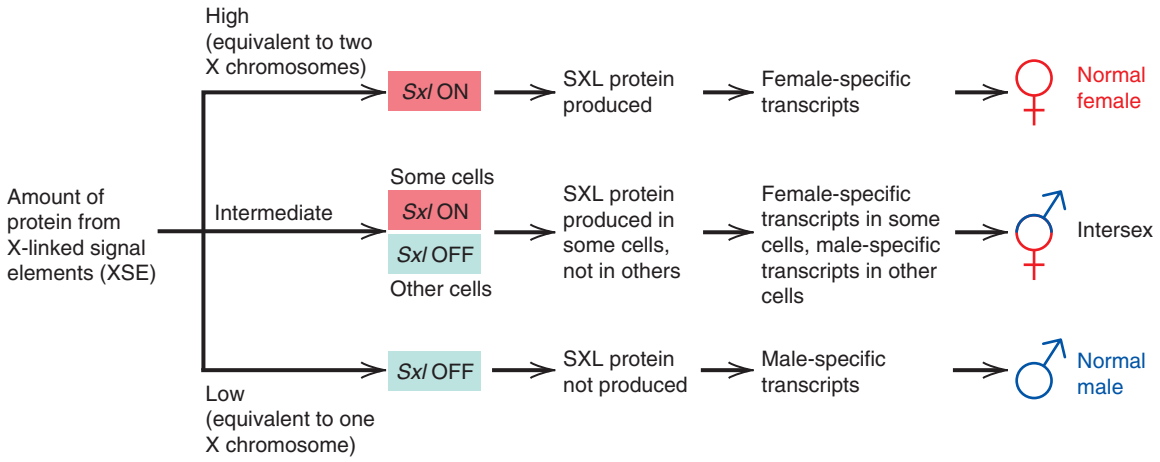


FIGURE 4.20 Early steps in the genetic control of sex determination in *Drosophila* through the activity of the Sex-lethal gene *Sxl*.

embryo that is a mosaic of male and female cells, and the phenotypic result is an intersex.

Sex determination in *Drosophila* is an excellent example of why correlation does not necessarily mean causation, and of the principle that ideas in science, even those that are long entrenched and widely believed, sometimes turn out to be wrong.

4.5 Probability in the Prediction of Progeny Distributions

Genetic transmission includes a large component of chance. A particular gamete from an *Aa* organism might or might not include the *A* allele, depending on chance. A particular gamete from an *Aa Bb* organism might or might not include both the *A* and *B* alleles, depending on the chance orientation of the chromosomes on the metaphase I plate. Genetic ratios result not only from the chance assortment of genes into gametes, but also from the chance combination of gametes into zygotes. Although exact predictions are not possible for any particular event, it is possible to determine the probability that a particular event will be realized, as we saw in Chapter 3. In this section, we consider some additional probability methods used in interpreting genetic data.

Using the Binomial Distribution in Genetics

The addition rule of probability deals with outcomes of a genetic cross that are mutually exclusive. Outcomes are "mutually exclusive" if they are incompatible in the sense that they cannot occur at the same time. For example, the possible sex distributions among three children consist of four mutually exclusive outcomes: zero, one, two, or three girls. These outcomes have probabilities 1/8, 3/8, 3/8, and 1/8, respectively. The addition rule states that the overall probability of any combination of mutually exclusive events is equal to the sum of the probabilities of the events taken separately. For example, the probability that a sibship of size three contains *at least one girl* includes the outcomes one, two, and three girls, so the overall probability of at least one girl equals $3/8 + 3/8 + 1/8 = 7/8$.

The multiplication rule of probability deals with outcomes of a genetic cross that are independent. Any two possible outcomes are independent if the knowledge that one outcome is actually realized provides no information about whether the other is realized also. For example, in a sequence of births, the sex of any particular child is not affected by the sex of any sibling born earlier and has no influence whatsoever on the distribution of sexes of any siblings born later. Each successive birth is independent of all the others. When possible outcomes are independent, the multiplication rule states that the probability of any combination of outcomes being realized equals the product of the probabilities of all the individual outcomes taken separately. For example, the probability that a sibship of three children will consist of three girls equals $1/2 \times 1/2 \times 1/2$, because the probability of each birth resulting in a girl is 1/2, and the successive births are independent.

Probability calculations in genetics frequently use the addition and multiplication rules together. For example, the probability that all three children in a family will be of the same sex uses both the addition and the multiplication rules. The probability that all three will be girls is $(1/2)(1/2)(1/2) = 1/8$, and the probability that all three will be boys is also 1/8. Because these outcomes are mutually exclusive (a sibship of size three cannot include three boys *and* three girls), the probability of either three girls or three boys is the sum of the two probabilities, or $1/8 + 1/8 = 1/4$. The other possible outcomes for sibships of size three are that two of the children will be girls and the other a boy, and that two will be boys and the other a girl. For each of these outcomes, three different orders of birth are possible—for example, GGB, GBG, and BGG—each having a probability of $1/2 \times 1/2 \times 1/2 = 1/8$. The probability of two girls and a boy, disregarding birth order, is the sum of the probabilities for the three possible orders, or 3/8; likewise, the probability of two boys and a girl is also 3/8. Therefore, the distribution of probabilities for the sex ratio in families with three children is

GGG	GGB GBG BGG	GBB BGB BBG	BBB

$$(1/2)^3 + 3(1/2)^2(1/2) + 3(1/2)(1/2)^2 + (1/2)^3 =$$
$$1/8 + 3/8 + 3/8 + 1/8 = 1$$

The sex ratio information in this display can be obtained more directly by expanding the binomial expression $(p + q)^n$, in which p is the probability of the birth of a girl (1/2), q is the probability of the birth of a boy (1/2), and n is the number of children. In the present example,

$$(p + q)^3 = 1p^3 + 3p^2q + 3pq^2 + 1q^3$$

in which the red numerals are the possible number of birth orders for each sex distribution. Similarly, the binomial distribution of

probabilities for the sex ratios in families of five children is

$$(p + q)^5 = 1p^5 + 5p^4q + 10p^3q^2 + 10p^2q^3 + 5pq^4 + 1q^5$$

Each term tells us the probability of a particular combination. For example, the third term is the probability of three girls (p^3) and two boys (q^2) in a family that has five children:

$$10(1/2)^3(1/2)^2 = 10/32 = 5/16$$

There are $n + 1$ terms in a binomial expansion to the power n. The exponents of p decrease by one from n in the first term to 0 in the last term, and the exponents of q increase by one from 0 in the first term to n in the last term. The coefficients generated by successive values of n can be arranged in a regular triangle known as **Pascal's triangle**, shown for $n = 0$ to 10 in FIGURE 4.21. The horizontal rows of the triangle are symmetrical; each row begins and ends with a 1, and each other entry can be obtained as the sum of the two numbers on either side of it in the row above.

To generalize just a bit, if the probability of event A is p, that of event B is q, and the two events are independent and mutually exclusive, then the probability that A will be realized four times and B two times (in a specified order) is, by the multiplication rule, p^4q^2. Usually we are interested in a combination of events regardless of their order, such as "four A and two B." In this case, we multiply the probability that the combination 4A : 2B will be realized in any specified order by the number of possible orders. The number of different combinations of six events, four of type A and two of type B, is given by the coefficient of p^4q^2 in the expansion of $(p\,\mathrm{A} + q\,\mathrm{B})^6$. This coefficient can be found in the row for $n = 6$ in Pascal's triangle, as the fifth entry from the left. (It is the fifth because the successive entries are the coefficients of p^0q^6, p^1q^5, p^2q^4, p^3q^3, p^4q^2, p^5q^1, and p^6q^0.) Hence, the overall probability of four realizations of A and two realizations of B in six trials is given by $15p^4q^2$.

The general rule for repeated trials of events with constant probabilities is as follows:

> If the probability of event A is p and the probability of the alternative event B is q, the probability that, in n trials, event A is realized s times and event B is realized t times is
>
> $$\frac{n!}{s!t!}p^sq^t \qquad (1)$$

in which $s + t = n$ and $p + q = 1$. The symbol $n!$ is read as "n factorial," and it stands for

n	Coefficients
0	1
1	1 1
2	1 2 1
3	1 3 3 1
4	1 4 6 4 1
5	1 5 10 10 5 1
6	1 6 15 20 15 6 1
7	1 7 21 35 35 21 7 1
8	1 8 28 56 70 56 28 8 1
9	1 9 36 84 126 126 84 36 9 1
10	1 10 45 120 210 252 210 120 45 10 1

FIGURE 4.21 Pascal's triangle. The numbers in the nth row are the coefficients of the terms in the expansion of the polynomial $(p + q)^n$.

Table 4.2 Factorials

n	$n!$	n	$n!$
0	1	8	40,320
1	1	9	362,880
2	2	10	3,628,800
3	6	11	39,916,800
4	24	12	479,001,600
5	120	13	6,227,020,800
6	720	14	87,178,291,200
7	5040	15	1,307,674,368,000

the product of all positive integers from 1 through n (that is, $n! = 1 \times 2 \times 3 \times \cdots \times n$). Values n factorial from $n = 0$ through $n = 15$ are given in TABLE 4.2. The value $0! = 1$ is defined arbitrarily to generalize its use in mathematical formulas. The magnitude of $n!$ increases very rapidly; 15! is more than a trillion.

Equation (1) applies even when either s or t equals 0 because $0! = 1$. (Remember also that any number raised to the zero power equals 1.) Any individual term in the expansion of the binomial $(p + q)^n$ is given by Equation (1) for the appropriate values of s and t. In Pascal's triangle, successive entries in the nth row are the values of $n!/(s!\,t!)$ for $s = 0, 1, 2, \ldots, n$.

Let us consider a specific application of Equation (1), in which we calculate the probability that a mating between two heterozygous parents will yield exactly the expected 3 : 1 ratio of the dominant and recessive traits among sibships of a particular size. The probability p of a child showing the dominant trait is 3/4, and the probability q of a child showing the recessive

trait is 1/4. Suppose we wanted to know how often families with eight children contain exactly six children with the dominant phenotype and two with the recessive phenotype. This is the "expected" Mendelian ratio. In this case, $n = 8, s = 6, t = 2$, and the probability of this combination of events is

$$\frac{8!}{6!\,2!}p^6q^2 = \frac{8!}{6! \times 2!}(3/4)^6(1/4)^2 = 0.31$$

That is, in only 31 percent of the families with eight children would the offspring exhibit the expected 3 : 1 phenotypic ratio; the other sibships would deviate in one direction or the other because of chance variation. The importance of this example is in demonstrating that although a 3 : 1 ratio is the "expected" outcome (and is also the single most likely outcome), the majority of the families (69 percent) actually have a distribution of offspring different from 3 : 1.

Meaning of the Binomial Coefficient

The factorial part of the binomial expansion in Equation (1), which equals $n!/(s!\,t!)$, is called the *binomial coefficient*. As we have noted, this ratio enumerates all possible ways in which s elements of one kind and t elements of another kind can be arranged in order, provided that the s elements and the t elements are not distinguished among themselves. A specific example might include s yellow peas and t green peas. Although the yellow peas and the green peas can be distinguished from each other because they have different colors, the yellow peas are not distinguishable from one another (because they are all yellow), nor are the green peas (because they are all green).

The reasoning behind the factorial formula begins with the observation that the total number of elements is $s + t = n$. Given n elements, each distinct from the next, the number of different ways in which they can be arranged is:

$$n \times (n-1) \times (n-2) \times \cdots \times 3 \times 2 \times 1$$

Why? Because the first element can be chosen in n ways, and once it is chosen, the next can be chosen in $n - 1$ ways (because only $n - 1$ are left to choose from), and once the first two are chosen, the third can be chosen in $n - 2$ ways, and so on. Finally, once $n - 1$ elements have been chosen, there is only one way to choose the last element. The $s + t$ elements can be arranged in $n!$ ways, provided that the elements are all distinguished among themselves. However, applying again the argument we just used, each of the $n!$ particular arrangements must include $s!$ different arrangements of the s elements and $t!$ different arrangements of the t elements, or $s! \times t!$ altogether. Dividing $n!$ by $s! \times t!$ therefore yields the binomial coefficient for the number of ways in which the s elements and the t elements can be arranged when the elements of each type are not distinguished among themselves.

4.6 Testing Goodness of Fit to a Genetic Hypothesis

Geneticists often need to decide whether an observed ratio is in satisfactory agreement with a theoretical prediction. Mere inspection of the data is unsatisfactory because different investigators may disagree. Suppose, for example, that we crossed a plant having purple flowers with a plant having white flowers and, among the progeny, observed 14 plants with purple flowers and 6 with white flowers. Is this result close enough to be accepted as a 1 : 1 ratio? What if we observed 15 plants with purple flowers and 5 with white flowers? Is this result consistent with a 1 : 1 ratio? There is bound to be statistical variation in the observed results from one experiment to the next. Who is to say what results are consistent with a particular genetic hypothesis? In this section, we describe a test of whether observed results deviate too far from a theoretical expectation. The test is called a test for **goodness of fit**, where the word *fit* means how closely the observed results "fit," or agree with, the expected results.

The Chi-Square Method

A conventional measure of goodness of fit is a value called **chi-square** (its symbol is χ^2), which is calculated from the number of progeny observed in each of various classes, compared with the number expected in each of the classes on the basis of some genetic hypothesis. For example, in a cross between plants with purple flowers and those with white flowers, we may be interested in testing the hypothesis that the parent with purple flowers is heterozygous for one pair of alleles determining flower color and that the parent with white flowers is homozygous recessive. Suppose further that we examine 20 progeny plants from the mating and find that 14 are purple and 6 are white. The procedure for testing this genetic hypothesis (or any

other genetic hypothesis) by means of the chi-square method is as follows:

1. ***State the genetic hypothesis in detail, specifying the genotypes and phenotypes of the parents and the possible progeny.*** In the example using flower color, the genetic hypothesis implies that the genotypes in the cross purple × white could be symbolized as *Pp* × *pp*. The possible progeny genotypes are *Pp* and *pp*.
2. ***Use the rules of probability to make explicit predictions of the types and proportions of progeny that should be observed if the genetic hypothesis is true. Convert the proportions to numbers of progeny (percentages are not allowed in a χ^2 test).*** If the hypothesis about the flower-color cross is true, then we should expect the progeny genotypes *Pp* and *pp* to occur in a ratio of 1 : 1. Because the hypothesis is that *Pp* flowers are purple and *pp* flowers are white, we expect the phenotypes of the progeny to be purple or white in the ratio 1 : 1. Among 20 progeny, the expected numbers are 10 purple and 10 white.
3. ***For each class of progeny in turn, subtract the expected number from the observed number. Square this difference and divide the result by the expected number.*** In our example, the calculation for the purple progeny is $(14 - 10)^2/10 = 1.6$, and that for the white progeny is $(6 - 10)^2/10 = 1.6$.
4. ***Sum the result of the numbers calculated in step 3 for all classes of progeny. The summation is the value of χ^2 for these data.*** The sum for the purple and white classes of progeny is $1.6 + 1.6 = 3.2$, and this is the value of χ^2 for the experiment, calculated on the assumption that our genetic hypothesis is correct.

In symbols, the calculation of χ^2 can be represented by the expression

$$\chi^2 = \sum \frac{(\text{Observed} - \text{Expected})^2}{\text{Expected}}$$

in which Σ means the summation over all the classes of progeny. Note that χ^2 is calculated using the observed and expected *numbers*, not the proportions, ratios, or percentages. Using something other than the actual numbers is the most common beginner's mistake in applying the χ^2 method. The χ^2 value is reasonable as a measure of goodness of fit, because the closer the observed numbers are to the expected numbers, the smaller the value of χ^2. A value of $\chi^2 = 0$ means that the observed numbers fit the expected numbers perfectly.

As another example of the calculation of χ^2, suppose that the progeny of an $F_1 \times F_1$ cross includes two contrasting phenotypes observed in the numbers 99 and 45. In this case the genetic hypothesis might be that the trait is determined by a pair of alleles of a single gene, in which case the expected ratio of dominant : recessive phenotypes among the F_2 progeny is 3 : 1. Considering the data, the question is whether the observed ratio of 99 : 45 is in satisfactory agreement with the expected 3 : 1. Calculation of the value of χ^2 is illustrated in **TABLE 4.3**. The total number of progeny is $99 + 45 = 144$. The *expected* numbers in the two classes, on the basis of the genetic hypothesis that the true ratio is 3 : 1, are calculated as $(3/4) \times 144 = 108$ and $(1/4) \times 144 = 36$. Because there are two classes of data, there are two terms in the χ^2 calculation:

$$\chi^2 = \frac{(99 - 108)^2}{108} + \frac{(45 - 36)^2}{36}$$
$$= 0.75 + 2.25$$
$$= 3.00$$

Once the χ^2 value has been calculated, the next step is to interpret whether this value represents a good fit or a bad fit to the expected

Table 4.3 Calculation of χ^2 for a monohybrid ratio

Phenotype (class)	Observed number	Expected number	Deviation from expected	$\frac{(\text{Deviation})^2}{\text{expected number}}$
Wildtype	99	108	−9	0.75
Mutant	45	36	+9	2.25
Total	144	144		$\chi^2 = 3.00$

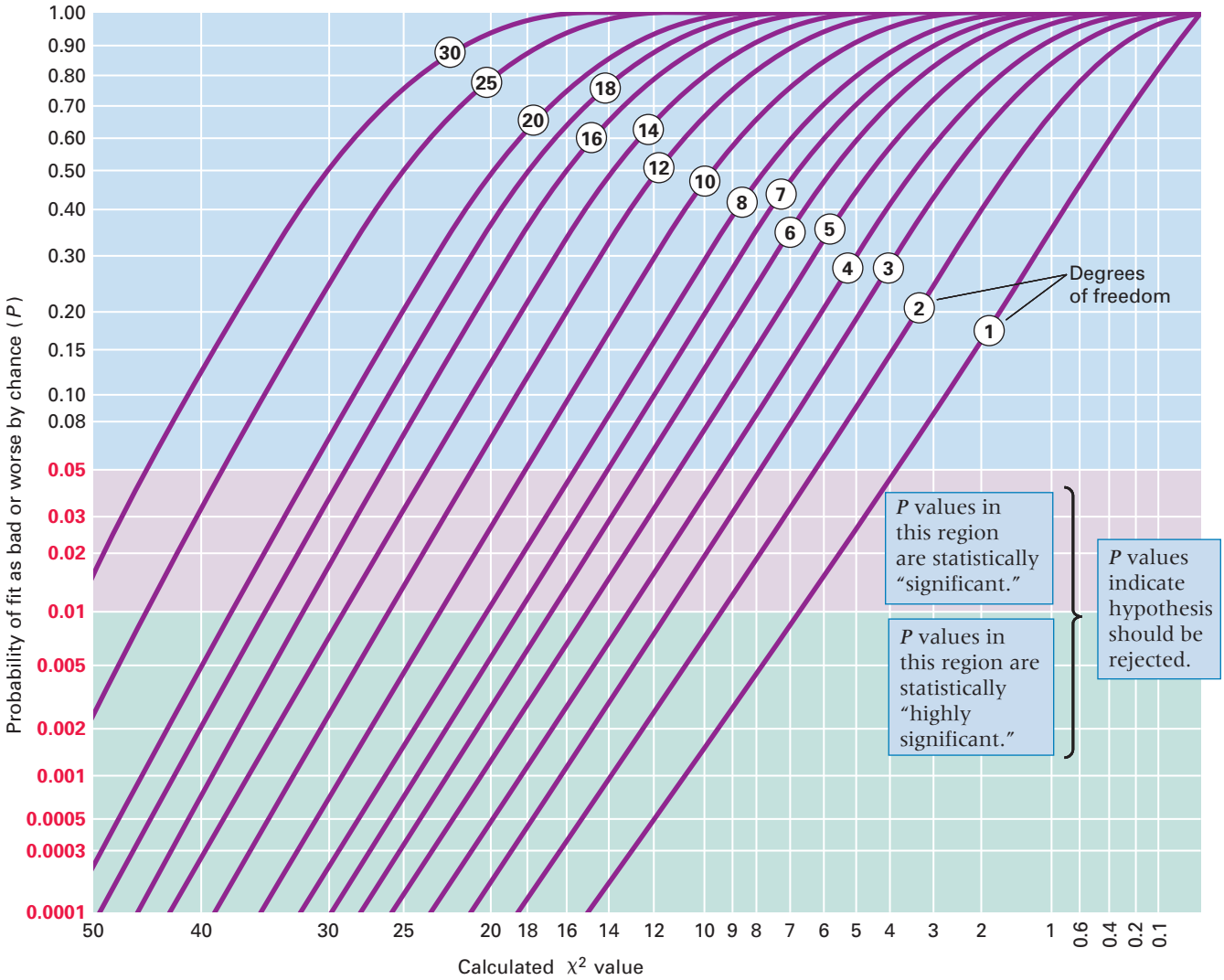


FIGURE 4.22 Graphs for interpreting goodness of fit to genetic predictions using the chi-square test. For any calculated value of χ^2 along the *x*-axis, the *y*-axis gives the probability *P* that chance alone would produce a fit as bad as or worse than that actually observed, when the genetic predictions are correct. Tests with *P* in the pink region (less than 5 percent) or in the green region (less than 1 percent) are regarded as statistically significant and normally require rejection of the genetic hypothesis that led to the prediction.

numbers. This assessment is done with the aid of the graphs in **FIGURE 4.22**. The *x*-axis gives the χ^2 values that reflect goodness of fit, and the *y*-axis gives the probability *P* that a worse fit (or one equally bad) would be obtained by chance, assuming that the genetic hypothesis is true. If the genetic hypothesis is true, then the observed numbers should be reasonably close to the expected numbers. Suppose that the observed χ^2 is so large that the probability of a fit as bad or worse is very small. Then the observed results do *not* fit the theoretical expectations. This means that the genetic hypothesis used to calculate the expected numbers of progeny must be rejected, because the observed numbers of progeny deviate too much from the expected numbers.

In practice, the critical values of *P* are conventionally chosen as 0.05 (the 5 percent level) and 0.01 (the 1 percent level). For *P* values ranging from 0.01 to 0.05, the probability that chance alone would lead to a fit as bad or worse is between 1 in 20 experiments and between 1 in 100, respectively. This is the middle region in Figure 4.22; if the *P* value falls in this range, the correctness of the genetic hypothesis is considered very doubtful. The result is said to be **statistically significant** at the 5 percent level. For *P* values smaller than 0.01, the probability that chance alone would lead to a fit as bad or worse is less than 1 in 100 experiments. This is the lower region in Figure 4.22; in this case, the result is said to be **highly significant** at the 1 percent level, and the genetic hypothesis is

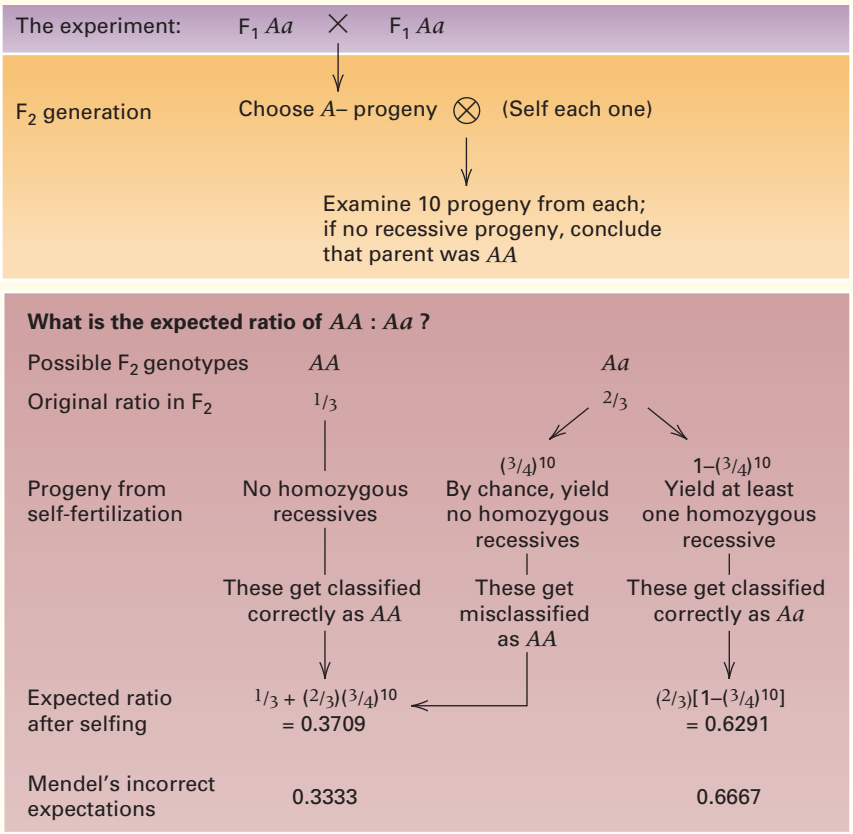
Seeds of Doubt

Ronald Aylmer Fisher 1936
University College,
London, England
Has Mendel's Work Been Rediscovered?

R. A. Fisher, one of the founders of modern statistics, was also interested in genetics. He gave Mendel's data a thorough going over and made an "abominable discovery." Fisher's unpleasant discovery was that some of Mendel's experiments yielded a better fit to the wrong expected values than they did to the right expected values. At issue are two series of experiments consisting of progeny tests in which F_2 plants with the dominant phenotype were self-fertilized and their progeny examined for segregation to ascertain whether each parent was heterozygous or homozygous. In the first series of experiments, Mendel explicitly states that he cultivated 10 seeds from each plant. What Mendel did not realize, apparently, is that inferring the genotype of the parent on the basis of the phenotypes of 10 progeny introduces a slight bias. The reason is shown in the accompanying illustration. Because a fraction $(3/4)^{10}$ of all progenies from a heterozygous parent will not exhibit segregation, purely as a result of chance, this proportion of Aa parents gets misclassified as AA. The expected proportion of "apparent" AA plants is $(1/3) + (2/3)(3/4)^{10}$ and that of Aa plants is $(2/3)[1 - (3/4)^{10}]$, for a ratio of 0.37 : 0.63. In the first series of experiments, among 600 plants tested, Mendel reports a ratio of 0.335 : 0.665, which is in better agreement with the incorrect expectation of 0.33 : 0.67 than with 0.37 : 0.63. In the second series of experiments, among 473 progeny, Mendel reports a ratio of 0.32 : 0.68, which is again in better agreement with 0.33 : 0.67 than with 0.37 : 0.63. Modern scholars have concluded that Fisher most likely misinterpreted Mendel's description of the second series of experiments, and that the results of Mendel's first series of experiments actually fit Fisher's expectation very well.

In connection with these tests of homozygosity by examining ten offspring formed by self-fertilization, it is disconcerting to find that the proportion of plants misclassified by this test is not inappreciable. Between 5 and 6 percent of the heterozygous plants will be classified as homozygous. . . . Now among 600 plants tested by Mendel 201 were classified as homozygous and 399 as heterozygous. . . . The deviation [from the true expected values of 222 and 378] is one to be taken seriously. . . . A deviation as fortunate as Mendel's is to be expected once in twenty-nine trials. . . . [In the second series of experiments], a total deviation of the magnitude observed, and in the right direction, is only to be expected once in 444 trials; there is therefore a serious discrepancy. . . . If we could suppose that larger progenies, say fifteen plants, were grown on this occasion, the greater part of the discrepancy would be removed. . . . Such an explanation, however, could not explain the discrepancy observed in the first group of experiments, in which the procedure is specified, without the occurrence of a coincidence of considerable improbability. . . . The reconstruction [of Mendel's experiments] gives no doubt whatever that his report is to be taken entirely literally, and that his experiments were carried out in just the way and much in the order that they are recounted. The detailed reconstruction of his programme on this assumption leads to no discrepancy whatsoever. A serious and almost inexplicable

The reconstruction [of Mendel's experiments] gives no doubt whatever that his report is to be taken entirely literally, and that his experiments were carried out in just the way and much in the order that they are recounted.



(continued on page 146)

Seeds of Doubt, cont.

discrepancy has, however, appeared, in that in two series of results the numbers observed agree excellently with the two to one ratio, which Mendel himself expected, but differ significantly from what should have been expected had his theory been corrected to allow for the small size of his test progenies. . . . Although no explanation can be expected to be satisfactory, it remains a possibility among others that Mendel was deceived by some assistant who knew too well what was expected.

Source: R. A. Fisher, *Ann. Sci.* 1 (1936): 115–137.

rejected outright. If the terminology of statistical significance seems backward, it is because the term *significant* refers to the magnitude of the deviation between the observed and the expected numbers; in a result that is statistically significant, there is a large ("significant") difference between what is observed and what is expected.

To use Figure 4.22 to determine the *P* value corresponding to a calculated χ^2, we need the number of **degrees of freedom** of the particular χ^2 test. For the type of χ^2 test illustrated in Table 4.3, the number of degrees of freedom equals the number of classes of data minus 1. Table 4.3 contains two classes of data (wildtype and mutant), so the number of degrees of freedom is $2 - 1 = 1$. The reason for subtracting 1 is that, in calculating the expected numbers of progeny, we make sure that the total number of progeny is the same as that actually observed. For this reason, one of the classes of data is not really "free" to contain any number we might specify; because the expected number in one class must be adjusted to make the total come out correctly, one "degree of freedom" is lost. Analogous χ^2 tests with three classes of data have 2 degrees of freedom, and those with four classes of data have 3 degrees of freedom.

Once we have determined the appropriate number of degrees of freedom, we can interpret the χ^2 value in Table 4.3. Refer to Figure 4.22, and observe that each curve is labeled with its degrees of freedom. To determine the *P* value for the data in Table 4.3, in which the χ^2 value is 3.00, first find the location of $\chi^2 = 3.00$ along the *x*-axis in Figure 4.22. Trace vertically on this line until you intersect the curve with 1 degree of freedom. Then trace horizontally to the left until you intersect the *y*-axis, and read the *P* value; in this case, $P = 0.08$. This means that chance alone would produce a χ^2 value as great as or greater than 3.00 in about 8 percent of experiments of the type in Table 4.3; and, because the *P* value is within the upper region, the goodness of fit to the hypothesis of a 3 : 1 ratio of wildtype : mutant is judged to be satisfactory.

As a second illustration of the χ^2 test, we will determine the goodness of fit of Mendel's round-versus-wrinkled data to the expected 3 : 1 ratio. Among the 7324 seeds that he observed, 5474 were round and 1850 were wrinkled. The expected numbers are $(3/4) \times 7324 = 5493$ round and $(1/4) \times 7324 = 1831$ wrinkled. The χ^2 value is calculated as:

$$\chi^2 = \frac{(5474 - 5493)^2}{5493} + \frac{(1850 - 1831)^2}{1831} = 0.26$$

The fact that the χ^2 is less than 1 already implies that the fit is very good. To find out how good, note that the number of degrees of freedom equals $2 - 1 = 1$ because there are two classes of data (round and wrinkled). From Figure 4.22, the *P* value for $\chi^2 = 0.26$ with 1 degree of freedom is approximately 0.65. This means that in about 65 percent of all experiments of this type, a fit as bad or worse would be expected simply because of chance. Only about 35 percent of all experiments would yield a better fit.

Are Mendel's Data Too Good to Be True?

Many of Mendel's experimental results are very close to the expected values. For the ratios listed in Table 3.1 in Chapter 3, the χ^2 values are 0.26 (round versus wrinkled seeds), 0.01 (yellow versus green seeds), 0.39 (purple versus white flowers), 0.06 (inflated versus constricted pods), 0.45 (green versus yellow pods), 0.35 (axial versus terminal flowers), and 0.61 (long versus short stems). (As an exercise in χ^2, you

should confirm these calculations for yourself.) All of the χ^2 tests have P values of 0.45 or greater (Figure 4.22), which means that the reported results are in excellent agreement with the theoretical expectations.

The statistician Ronald Fisher pointed out in 1936 that Mendel's results are *suspiciously* close to the theoretical expectations. In a large number of experiments, some experiments can be expected to yield fits that appear doubtful simply because of chance variation from one experiment to the next. In Mendel's data, the doubtful values that are to be expected appear to be missing. **FIGURE 4.23** shows the observed deviations in Mendel's experiments compared with the deviations expected by chance. (The measure of deviation is the square root of the χ^2 value, assigned either a plus or a minus sign according to whether the dominant or the recessive phenotypic class was in excess of the expected number.) For each magnitude of deviation, the height of the bar on the right gives the number of experiments that Mendel observed with such a magnitude of deviation, and that of the bar on the left gives the number of experiments expected to deviate by this amount as a result of chance alone. There are clearly too few experiments with deviations smaller than −1 or larger than +1. This type of discrepancy could be explained if Mendel discarded or repeated a few experiments with large deviations that made him suspect that the results were not to be trusted.

Did Mendel cheat? Did he deliberately falsify his data to make them appear better? Mendel's paper reports extremely deviant ratios from individual plants, as well as experiments repeated when the first results were doubtful. These are not the kinds of things a dishonest person would admit. Only a small bias is necessary to explain the excessive goodness of fit in Figure 4.23. In a count of seeds or individual plants, only about 2 phenotypes per 1000 would need to be assigned to the wrong category to account for the bias in the 91 percent of the data generated by the testing of monohybrid ratios. The excessive fit could also be explained if three or four entire experiments were discarded or repeated because deviant results were attributed to pollen contamination or other accident. After careful reexamination

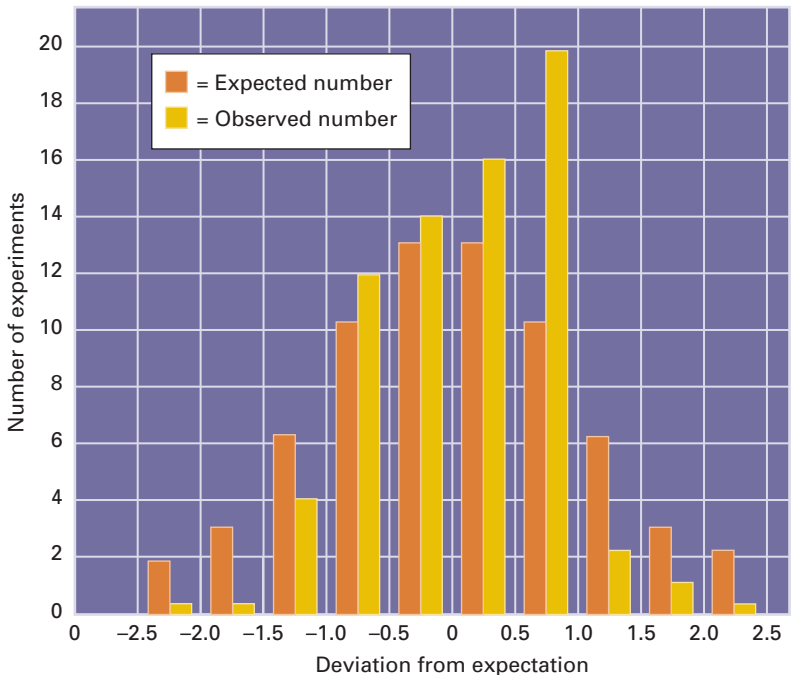


FIGURE 4.23 Distribution of deviations observed in 69 of Mendel's experiments (yellow bars) compared with expected values (orange bars). There is no suggestion that the data in the middle have been adjusted to improve the fit, but there are fewer experiments with large deviations than might be expected. Several experiments with large deviations may have been discarded or repeated.

of Mendel's data in 1966, the evolutionary geneticist Sewall Wright concluded,

> Mendel was the first to count segregants at all. It is rather too much to expect that he would be aware of the precautions now known to be necessary for completely objective data. . . . Checking of counts that one does not like, but not of others, can lead to systematic bias toward agreement. I doubt whether there are many geneticists even now whose data, if extensive, would stand up wholly satisfactorily under the χ^2 text. . . . Taking everything into account, I am confident that there was no deliberate effort at falsification.

Mendel's data are some of the most extensive and complete "raw data" ever published in genetics. Additional examinations of the data will surely be carried out as new statistical approaches are developed. However, the principal point to be emphasized is that up to the present time, no reputable statistician has alleged that Mendel knowingly and deliberately adjusted his data in favor of the theoretical expectation.

CHAPTER SUMMARY

- Chromosomes in eukaryotic cells are usually present in pairs.
- The chromosomes of each pair separate in meiosis, one going to each gamete.
- In meiosis, the chromosomes of different pairs undergo independent assortment because nonhomologous chromosomes move independently.
- In many animals, sex is determined by a special pair of chromosomes, the X and Y.
- The "criss-cross" pattern of inheritance of X-linked genes is determined by the fact that a male receives his X chromosome only from his mother and transmits it only to his daughters.
- Irregularities in the inheritance of an X-linked gene in *Drosophila* gave experimental proof of the chromosomal theory of heredity.
- The progeny of genetic crosses follow the binomial probability formula.
- The chi-square statistical test is used to determine how well the observed genetic data agree with the expectations derived from a hypothesis.

REVIEW THE BASICS

- What is the genetic significance of the fact that gametes contain half the chromosome complement of somatic cells?
- The term *mitosis* derives from the Greek *mitos*, which means "thread." The term *meiosis* derives from the Greek *meioun*, which means "to make smaller." What feature, or features, of these types of nuclear division might have led to the choice of these terms?
- Explain the meaning of the terms *reductional division* and *equational division*. What is reduced or kept equal? To which nuclear divisions do the terms refer?
- Explain the following statement: "Independent alignment of nonhomologous chromosomes at metaphase I of meiosis is the physical basis of independent assortment of genes in different chromosomes."
- What are some of the important differences between the first meiotic division and the second meiotic division?
- Draw a diagram of a bivalent and label the following parts: centromere, sister chromatids, nonsister chromatids, homologous chromosomes, chiasma.
- What are the principal characteristics of human pedigrees in which a rare X-linked recessive allele is segregating?
- In what ways is the inheritance of a Y-linked gene different from that of an X-linked gene?
- How did nondisjunction "prove" the chromosome theory of heredity?
- Why is a statistical test necessary to determine whether an observed set of data yields an acceptable fit to the result expected from a particular genetic hypothesis?
- What statistical test is often used for this purpose?
- What are the conventional *P* values for significant and highly significant, and what do these numbers mean?

GUIDE TO PROBLEM SOLVING

PROBLEM 1 A recessive mutation in an X-linked gene results in hemophilia, marked by a prolonged increase in the time needed for blood to clot. Suppose that two phenotypically normal parents produce three normal daughters and a son affected with hemophilia.

(a) What is the probability that all of the daughters are heterozygous carriers?

(b) If one of the daughters mates with a normal male and produces a son, what is the probability that the son will be affected?

Answer

(a) Because the phenotypically normal parents have an affected son who gets his only X chromosome from his mother, the mother must be a carrier of the mutation. Therefore, the probability that any particular daughter is a carrier is $\frac{1}{2}$. The probability that all three daughters are carriers is $(\frac{1}{2})^3$ because their births are independent events.

(b) If the daughter is not a carrier, the probability of an affected son is 0; and if the daughter is a carrier, the probability of an affected son is $\frac{1}{2}$. Because the probability of the daughter being a carrier is $\frac{1}{2}$ (part **a**), the overall probability of an affected son is $(\frac{1}{2}) \times 0 + (\frac{1}{2}) \times (\frac{1}{2}) = \frac{1}{4}$.

PROBLEM 2 In a sibship with seven children, what is the probability that it includes four boys and three girls or three boys and four girls? Assume that each child has an equal likelihood of being a boy or a girl.

Answer The probability that the sibship consists of four boys and three girls, in any order of birth, equals $[7!/(4!3!)](\frac{1}{2})^4(\frac{1}{2})^3$, where the factor in square brackets is the number of possible birth orders of four boys and three girls. This probability works out to $^{35}/_{128}$. Likewise, the probability that the sibship consists of three boys and four girls is $[7!/(3!4!)](\frac{1}{2})^3(\frac{1}{2})^4$, which also equals $^{35}/_{128}$. The question asks for the probability of either four boys and three girls or three boys and four girls, and these events are mutually exclusive; hence the overall probability is $^{35}/_{128} + {}^{35}/_{128} = {}^{35}/_{64}$, or about 55%.

PROBLEM 3 A geneticist carries out a cross between two strains of fruit flies that are heterozygous for a recessive allele of each of two genes, *st* (scarlet) and *bw* (brown), affecting eye color. Flies homozygous for *st* have bright red (scarlet) eyes,

whereas st^+/st^+ and st^+/st genotypes have wildtype brick-red eyes. Flies homozygous for *bw* have brown eyes, whereas $bw^+/\ bw^+$ and $bw^+/\ b$ genotypes have wildtype brick-red eyes. The double homozygous genotype *st/st*; *bw/bw* results in white eyes. In a test of independent assortment of these two genes, a geneticist crosses st^+/st; bw^+/bw females with st^+/st; bw^+/bw males. Among 240 progeny there are 150 flies with wildtype eyes, 36 with scarlet eyes, 46 with brown eyes, and 8 with white eyes.

(a) Under the null hypothesis that these genes undergo independent assortment, what are the expected numbers in each phenotypic class?

(b) What is the value of the chi-square in a test of goodness of fit between the observed values and the expected values based on the null hypothesis of independent assortment?

(c) How many degrees of freedom does this chi-square value have?

(d) What is the *P*-value for the chi-square value in this goodness-of-fit test? Does this *P*-value support the null hypothesis of independent assortment or should the hypothesis be rejected?

(e) Would a greater chi-square value increase or decrease the *P*-value?

Answer

(a) Because this is a dihybrid cross st^+/st; $bw^+/bw \times st^+/st$; bw^+/bw, the ratio of 9 : 3 : 3 : 1 of the offspring phenotypes should be expected if the genes assort independently. We can calculate the expected number of flies in each class of the progeny:

Phenotype	Progeny	Expected number
wildtype	$st^+/-$; $bw^+/-$	(9/16) × 240 = 135
scarlet	*st/st*; $bw^+/-$	(3/16) × 240 = 45
brown	$st^+/-$; *bw/bw*	(3/16) × 240 = 45
white	*st/st*; *bw/bw*	(1/16) × 240 = 15

(b) The chi-square is calculated as

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

where the sum is over all classes of progeny. In this case,

$$\chi^2 = \frac{(150-135)^2}{135} + \frac{(36-45)^2}{45} + \frac{(46-45)^2}{45} + \frac{(8-15)^2}{15}$$

$$= 6.76$$

(c) The number of degrees of freedom equals the number of classes of data minus 1. In this case there are four classes of data; thus there are three degrees of freedom.

(d) The *P*-value for a chi-square value of 6.76 with 3 degrees of freedom equals 0.08. This *P*-value is greater than 0.05. Therefore, we should not reject the null hypothesis of independent assortment.

(e) A greater chi-square value would decrease the *P*-value.

PROBLEM 4 The mutation for bar-shaped eyes in *Drosophila melanogaster* shows the following features of genetic transmission:

(a) The mating of bar-eyed males with wildtype females produces wildtype sons and bar-eyed daughters.

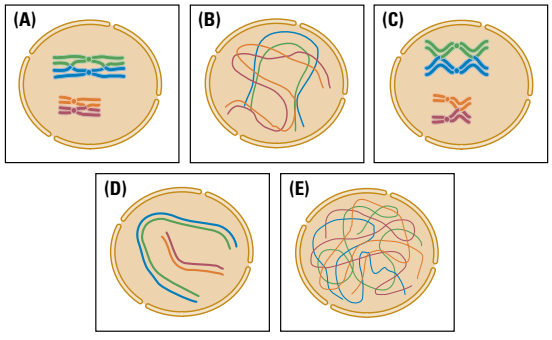
(b) The bar-eyed females from the mating in part **a**, when mated with wildtype males, yield a 1 : 1 ratio of bar : wildtype sons and a 1 : 1 ratio of bar : wildtype daughters.

What mode of inheritance do these characteristics suggest?

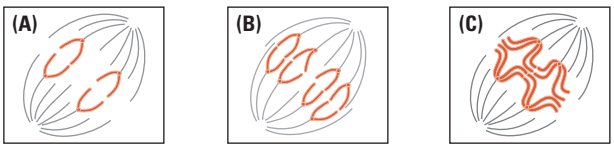
Answer Because the sexes are affected unequally in the progeny of the mating in **a**, some association with the sex chromosomes is suggested. The progeny from mating **a** provide the important clue. Because a male receives his X-chromosome from his mother, then the observation that all males are wildtype suggests that the gene for bar eyes is on the X-chromosome. The fact that all daughters are affected is also consistent with X-linkage, provided that the *bar* mutation is dominant. Mating **b** confirms the hypothesis of a dominant X-linked gene, because the females from a mating **a** would have the genotype *bar*/+ and so would produce the observed progeny. The data are therefore consistent with the *bar* mutation being an X-linked dominant.

ANALYSIS AND APPLICATIONS

4.1 The diagrams below illustrate a pair of homologous chromosomes in prophase I of meiosis. Which diagram corresponds to each stage: leptotene, zygotene, pachytene, diplotene, and diakinesis?



4.2 The diagrams below illustrate anaphase in an organism that has two pairs of homologous chromosomes. Identify the stages as mitosis, meiosis I, or meiosis II.

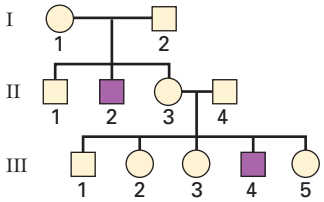


4.3 What is the probability that four offspring from the mating *Aa* × *Aa* consist of exactly 3 *A*− and 1 *aa*?

4.4 A woman who is heterozygous for both a phenylketonuria mutation and an X-linked hemophilia mutation has a child with a phenotypically normal man who is also heterozygous for a phenylketonuria

mutation. What is the probability that the child will be affected by both diseases? (Assume that they are equally likely to have a boy or a girl.)

4.5 In the accompanying pedigree the purple symbols represent individuals affected with X-linked Duchenne muscular dystrophy. What is the probability that the woman III-3 is a heterozygous carrier?



4.6 A chromosomally normal woman and a chromosomally normal man have a son whose sex-chromosome constitution is XYY. In which parent, and in which meiotic division, did the nondisjunction take place?

4.7 *Drosophila virilis* is a diploid organism with 6 pairs of chromosomes (12 chromosomes altogether). How many chromatids and chromosomes are present in the following stages of cell division:

(a) Metaphase of mitosis?
(b) Metaphase I of meiosis?
(c) Metaphase II of meiosis?

4.8 In a trihybrid cross with genes that undergo independent assortment:

(a) What is the expected proportion of triply heterozygous offspring in the F_2 generation?
(b) What is the expected proportion of triply homozygous offspring in the F_2 generation?

4.9 The diploid gekkonid lizard *Gonatodes taniae* from Venezuela has a somatic chromosome number of 16. If the centromeres of the 8 homologous pairs are designated as *Aa, Bb, Cc, Dd, Ee, Ff, Gg,* and *Hh*:

(a) How many different combinations of centromeres could be produced during meiosis?
(b) What is the probability that a gamete will contain only those centromeres designated by capital letters?

4.10 Among sibships consisting of six children, and assuming a sex ratio of 1 : 1:

(a) What is the proportion with no girls?
(b) What is the proportion with exactly one girl?
(c) What is the proportion with exactly two girls?
(d) What is the proportion with exactly three girls?
(e) What is the proportion with three or more boys?

4.11 A litter of cats includes eight kittens. What is the probability that it contains an even number each of males and females? Assume an equal likelihood of male and female kittens, and for purposes of this problem regard 0 as an even number. Does the answer surprise you? Why?

4.12 A horticulturalist crossed a true-breeding onion plant with red bulbs to a true-breeding onion plant with white bulbs. All of the F_1 plants had white bulbs. When seeds resulting from self-fertilization of the F_1 plants were grown, onion bulbs were recovered in the ratio of 12 white bulbs : 3 red bulbs : 1 yellow bulb. Propose a hypothesis to explain these observations.

4.13 Among 160 progeny in the F_2 generation of a dihybrid cross, a geneticist observes four distinct phenotypes in the ratio 91: 21 : 37 : 11. She believes this result may be consistent with a ratio of 9 : 3 : 3 : 1. To test this hypothesis, she calculates the chi-square value. Does the test support her hypothesis, or should she reject it?

4.14 What is the value of the chi-square that tests goodness of fit between the observed numbers 40 : 60 as compared with the expected numbers 50 : 50?

4.15 What is the probability that a sibship of seven children includes at least one boy and at least one girl? Assume that a sex ratio is 1 : 1.

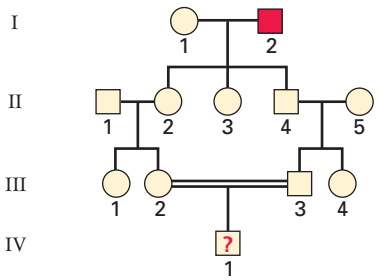
4.16 How many genotypes are possible for an autosomal gene with six alleles? How many genotypes are possible with an X-linked gene with six alleles?

4.17 The growth habit of the Virginia groundnut *Arachis hypogaea* can be "runner" (spreading) or "bunch" (compact). Two pure-breeding strains of groundnuts with the bunch growth habit are crossed. The F_1 plants have the runner growth habit. If the plants are allowed to self-fertilize, the F_2 progeny ratio is 9 runner: 7 bunch. What genetic hypothesis can account for these observations?

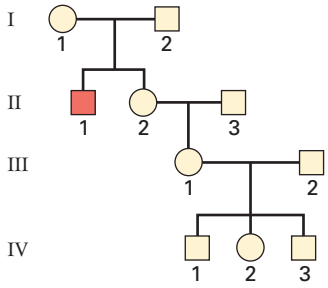
4.18 In some human pedigrees, the blue/brown eye color variation segregates like a single-gene difference with the brown allele dominant to the blue allele. Two brown-eyed individuals each of whom had a blue-eyed parent mate. Assuming that the trait segregates like a single-gene difference in this pedigree:

(a) What is the probability that the first child will have brown eyes?
(b) If a brown-eyed child results, what is the probability that the child will be heterozygous?
(c) What is the probability that this couple will have three children with blue eyes? That none of the three children will have blue eyes?

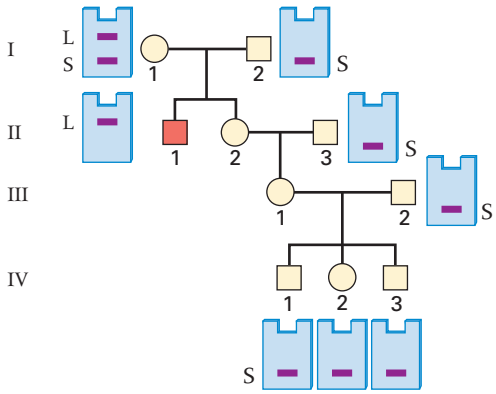
4.19 In the pedigree below, the male I-2 is affected with red-green color blindness owing to an X-linked mutation. What is the probability that male IV-1 is color blind? (Assume that the only possible source of the color blindness mutation in the pedigree is that from male I-2.)



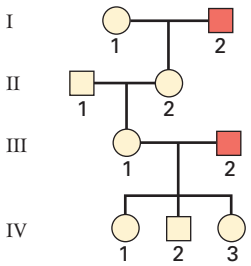
4.20 The male II-1 in the pedigree shown here is affected with a trait due to a rare X-linked recessive allele. Use the principles of conditional probability to calculate the probability that the female III-1 is a heterozygous carrier.



4.21 The pedigree shown below is the same as that in Problem 4.20 but with additional information based on molecular analysis. The male II-1 is again affected with a trait due to a rare X-linked recessive allele. The bands in the gel are restriction fragments that identify the wildtype allele (the shorter fragment S corresponding to the band near the bottom of the gel) or the mutant allele (the longer fragment L corresponding to the band near the top of the gel). From the information given in the pedigree and the gels, calculate the probability that the female III-1 is a heterozygous carrier.



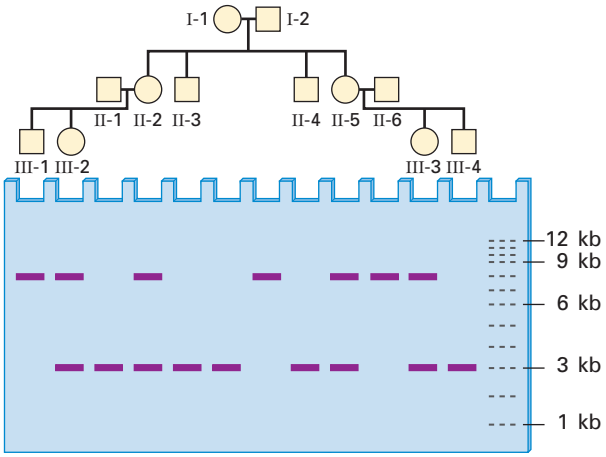
4.22 In the pedigree shown here, the males I-2 and III-2 are affected with a trait due to a common X-linked recessive allele. What is the probability that individual III-1 is heterozygous?



4.23 Yellow body color in *Drosophila* is determined by the recessive allele *y* of an X-linked gene, and the wildtype gray body color is determined by the y^+ allele. What genotype and phenotype ratios would be expected from the following crosses?

(a) yellow male × wildtype female
(b) yellow female × wildtype male
(c) daughter from mating in part **a** × wildtype male
(d) daughter from mating in part **a** × yellow male

4.24 The accompanying pedigree and gel diagram show the molecular phenotypes for an RFLP with two alleles. What mode of inheritance does the pedigree suggest? Based on this hypothesis, and using A_1 to represent the RFLP allele associated with the 3-kb band and A_2 to represent that associated with the 8-kb band, deduce the genotype of each individual in the pedigree.



4.25 Tall, yellow-flowered crocus is mated with short, white-flowered crocus. Both varieties are true breeding. All the F_1 plants are backcrossed with the short, white-flowered variety. This backcross yielded 800 progeny in the proportions 234 tall yellow, 203 tall white, 175 short yellow, and 188 short white plants. Does the observed result fit the genetic hypothesis of 1 : 1 : 1 : 1 segregation as assessed by a χ^2 test?

4.26 Attached-X chromosomes in *Drosophila* are formed from two X chromosomes attached to a common centromere. Females of genotype C(1)RM/Y, in which C(1)RM denotes the attached-X chromosomes, produce C(1)RM-bearing and Y-bearing gametes in equal proportions. What progeny is expected to result from the mating between a male carrying the X-linked allele, *y*, for yellow body and an attached-X female with wildtype body? How does this result differ from the typical pattern of X-linked inheritance? (Note: *Drosophila* zygotes containing three X chromosomes or no X chromosomes do not survive.)

4.27 A rare dominant autosomal mutation *W* results in wooly, curled hair in some European pedigrees. A woman with wooly hair with blood group O marries a man with straight hair (*ww*) with blood group AB. The genes are in different chromosomes.

(a) What are the chances that the mating will produce a wooly-haired group B child?
(b) What are the chances that the mating will produce a straight-haired group B child?
(c) If three straight-haired children with blood group A children are born to these parents, what are

the chances that the next child born will be wooly-haired and blood group B?

4.28 Ducklings of the domesticated mallard *Anas platyrhynchos* may exhibit dark brown dorsal plumage known as mallard, almost black dorsal plumage known as dusky, or a pattern known as restricted in which the black is confined to patches on the head and tail. These phenotypes result from the action of three alleles of a single autosomal gene. Three types of crosses were made, with the following results:

1. Restricted × mallard: all F_1 are restricted; crosses of $F_1 \times F_1$ yield an F_2 generation with the ratio 3 restricted : 1 mallard.
2. Mallard × dusky: all F_1 are mallard; crosses of $F_1 \times F_1$ produce an F_2 generation with the ratio 3 mallard : 1 dusky.
3. Restricted × dusky: all F_1 are restricted; crosses of $F_1 \times F_1$ produce an F_2 generation with the ratio 3 restricted : 1 dusky.

(a) Assume that an F_1 male from cross 1 is mated with an F_1 female from cross 2. List the phenotypes and their expected frequencies among the progeny of this cross.

(b) Assume that an F_1 male from cross 3 is mated with an F_1 female from cross 2. List the phenotypes and their expected frequencies among the progeny of this cross.

4.29 In cattle, an allele for the absence of horns shows complete dominance: *HH* and *Hh* are hornless or polled, and *hh* is horned. On the other hand, the effect of the allele producing red coat (*R*) shows incomplete dominance with the allele producing white coat color (*r*). The heterozygous genotype *Rr* is roan colored, an intermediate color in which white hairs are intermixed with red hairs. *H* and *R* undergo independent assortment.

(a) What would be the phenotype of an F_1 offspring from the mating *RR HH* × *rr hh*?

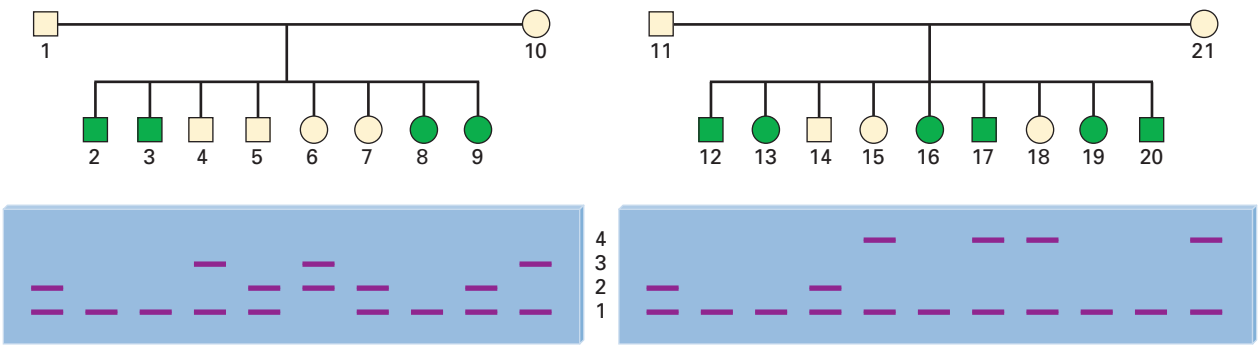
(b) What would be the phenotypes and their expected proportions in the F_2 progeny of the cross $F_1 \times F_1$ from part **a**?

(c) What would be the phenotypes and their expected proportions among the progeny derived from crossing F_1 individuals from part **a** to the original horned white stock?

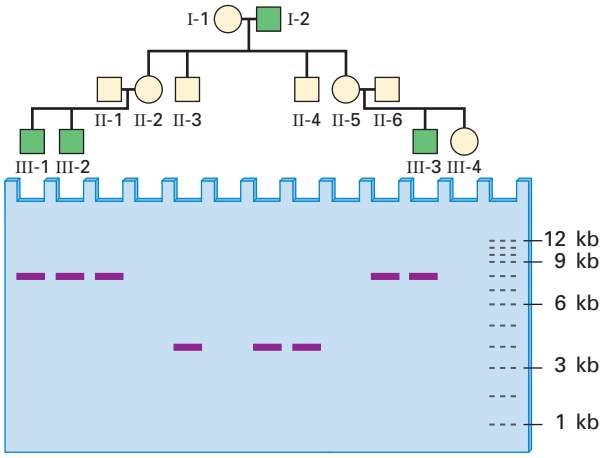
4.30 Familial Mediterranean fever (FMF) is a hereditary inflammatory disorder that affects groups of patients originating from around the Mediterranean Sea, especially Armenian and non-Ashkenazi Jewish populations, Anatolian Turks, and Levantine Arabs. FMF is inherited as an autosomal recessive. It is not known whether the gene causing the disease is the same in these different populations. To investigate this possibility, linkage of the allele to a series of VNTR (variable number of tandem repeat) polymorphisms was examined. One VNTR in chromosome 16 was especially informative. For this VNTR, two extended Armenian families from the same geographic region gave the results shown in the accompanying pedigrees and gels. The green symbols represent affected individuals. The numbers between the gels correspond to the VNTR alleles.

(a) Is there evidence for linkage of the allele for FMF to any VNTR allele?

(b) Do any of the data conflict with your explanation? If so, how do you account for it?

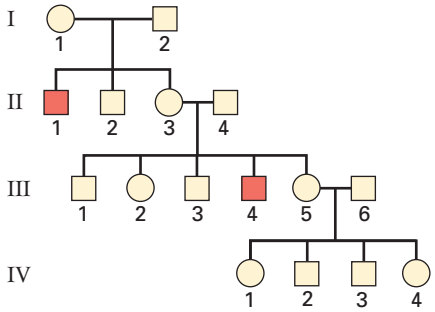


CHALLENGE PROBLEM 1 The accompanying pedigree and gel diagram pertain to a morphological phenotype (green symbols in the pedigree) and a molecular phenotype (a two-allele RFLP). What mode of inheritance do these data suggest for each trait?



CHALLENGE PROBLEM 2 In mice, the dominant *T* allele results in a short tail, but the homozygous *T*/*T* genotype is lethal. A cross between two short-tailed mice produces a litter of five pups. What is the expected distribution of tailless to tailed mice? Why are the two most likely outcomes equally probable?

CHALLENGE PROBLEM 3 X-linked hemophilia is present in the pedigree illustrated here with red symbols. What is the probability that the woman III-5 is a carrier, taking into account the information that she has had two normal sons? What is the probability that her next son will be affected?



GENETICS *on the web*

GeNETics on the Web will introduce you to some of the most important sites for finding genetics information on the Internet. To explore these sites, visit the Jones & Bartlett companion site to accompany *Genetics: Analysis of Genes and Genomes, Eighth Edition* at http://biology.jbpub.com/book/genetics/8e/.

There you will find a chapter-by-chapter list of highlighted keywords. When you select one of the keywords, you will be linked to a Web site containing information related to that keyword.