A. Mendelian Principles: Dominance, Segregation, independent assortment, deviation from Mendelian inheritance.

Mendel: The Father of Genetics

In 1902, the basic principles of genetics, which Archibald Garrod successfully applied to the inheritance of alkaptonuria, had just become widely known among biologists. Surprisingly, these principles had been discovered some 35 years earlier by Johann Gregor Mendel (1822–1884). Mendel was born in what is now part of the Czech Republic. Although his parents were simple farmers with little money, he was able to achieve a sound education and was admitted to the Augustinian monastery in Brno in September 1843. After graduating from seminary, Mendel was ordained a priest and appointed to a teaching position in a local school. He excelled at teaching, and the abbot of the monastery recommended him for further study at the University of Vienna, which he attended from 1851 to 1853. There, Mendel enrolled in the newly opened Physics Institute and took courses in mathematics, chemistry, entomology, paleontology, botany, and plant physiology. It was probably here that Mendel acquired the scientific method, which he later applied so successfully to his genetics experiments.

After 2 years of study in Vienna, Mendel returned to Brno, where he taught school and began his experimental work with pea plants. He conducted breeding experiments from 1856 to 1863 and presented his results publicly at meetings of the Brno Natural Science Society in 1865. Mendel's paper from these lectures was published in 1866. In spite of widespread interest in heredity, the effect of his research on the scientific community was minimal. At the time, no one seems to have noticed that Mendel had discovered the basic principles of inheritance.

In 1868, Mendel was elected abbot of his monastery, and increasing administrative duties brought an end to his teaching and eventually to his genetics experiments. He died at the age of 61 on January 6, 1884, unrecognized for his contribution to genetics.

The significance of Mendel's discovery was unappreciated until 1900, when three botanists—Hugo de Vries, Erich von Tschermak, and Carl Correns—began independently conducting similar experiments with plants and arrived at conclusions similar to those of Mendel. Coming across Mendel's paper, they interpreted their results in terms of his principles and drew attention to his pioneering work.

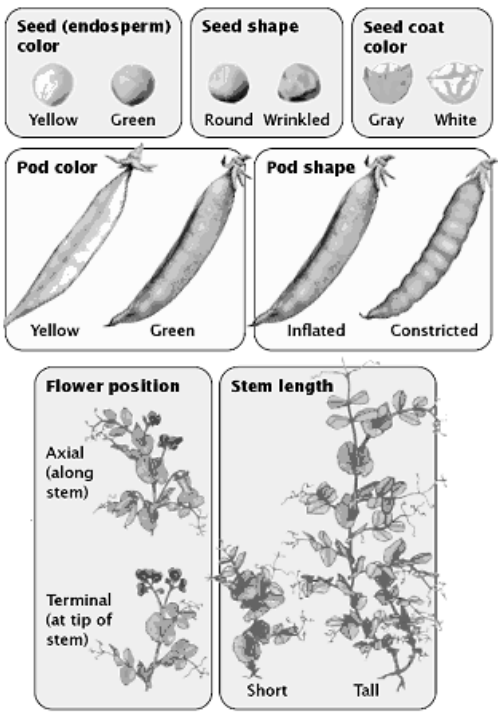
Mendel's Success

Mendel's approach to the study of heredity was effective for several reasons. Foremost was his choice of experimental subject, the pea plant Pisum sativum (FIGURE 1), which offered clear advantages for genetic investigation. It is easy to cultivate, and Mendel had the monastery garden and greenhouse at his disposal. Peas grow relatively rapidly, completing an entire generation in a single growing season. By today's standards, one generation per year seems frightfully slow— fruit flies complete a generation in 2 weeks and bacteria in 20 minutes—but Mendel was under no pressure to publish quickly and was able to follow the inheritance of individual characteristics for several generations. Had he chosen to work on an organism with a longer generation time—horses, for example—he might never have discovered the basis of inheritance.

Pea plants also produce many offspring—their seeds—which allowed Mendel to detect meaningful mathematical ratios in the traits that he observed in the progeny.

The large number of varieties of peas that were available to Mendel was also crucial, because these varieties differed in various traits and were genetically pure. Mendel was therefore able to begin with plants of variable, known genetic makeup.

Much of Mendel's success can be attributed to the seven characteristics that he chose for study (see Figure 1). He avoided characteristics that display a range of variation; instead, he focused his attention on those that exist in two easily differentiated forms, such as white versus gray seed coats, round versus wrinkled seeds, and inflated versus constricted pods.



1 **Mendel used the pea plant *Pisum sativum* in his studies of heredity.**

Finally, Mendel was successful because he adopted an experimental approach. Unlike many earlier investigators who just described the results of crosses, Mendel formulated hypotheses based on his initial observations and then conducted additional crosses to test his hypotheses. He kept careful records of the numbers of progeny possessing each type of trait and computed ratios of the different types. He paid close attention to detail, was adept at seeing patterns in

detail, and was patient and thorough, conducting his experiments for 10 years before attempting to write up his results.

Genetic Terminology

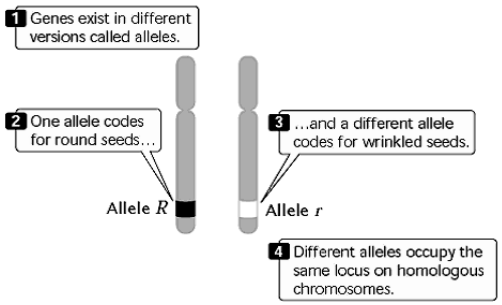
Before we examine Mendel's crosses and the conclusions that he made from them, it will be helpful to review some terms commonly used in genetics (Table 1). The term gene was a word that Mendel never knew. It was not coined until 1909, when the Danish geneticist Wilhelm Johannsen first used it. The definition of a gene varies with the context of its use, and so its definition will change as we explore different aspects of heredity. For our present use in the context of genetic crosses, we will define a gene as an inherited factor that determines a characteristic.

Table 1 Summary of important genetic terms

Term	Definition
Gene	A genetic factor (region of DNA) that helps determine a characteristic
Allele	One of two or more alternate forms of a gene
Locus	Specific place on a chromosome occupied by an allele
Genotype	Set of alleles that an individual possesses
Heterozygote	An individual possessing two different alleles at a locus
Homozygote	An individual possessing two of the same alleles at a locus
Phenotype or trait	The appearance or manifestation of a character
Character or characteristic	An attribute or feature

Genes frequently come in different versions called alleles (FIGURE 2). In Mendel's crosses, seed shape was determined by a gene that exists as two different alleles: one allele codes for round seeds and the other codes for wrinkled seeds. All alleles for any particular gene will be found at a specific place on a chromosome called the locus for that gene. (The plural of locus is loci; it's bad form in genetics— and incorrect—to speak of locuses.) Thus, there is a specific place—a locus—on a chromosome in pea plants where the shape of seeds is determined. This locus might be occupied by an allele for round seeds or one for wrinkled seeds. We will use the term allele when referring to a specific version of a gene; we will use the term gene to refer more generally to any allele at a locus. The genotype is the set of alleles that an individual organism possesses. A diploid organism that possesses two identical alleles is homozygous for that locus. One that possesses two different alleles is heterozygous for the locus.

Another important term is phenotype, which is the manifestation or appearance of a characteristic. A phenotype can refer to any type of characteristic: physical, physiological, biochemical, or behavioral. Thus, the condition of having round seeds is a phenotype, a body weight of 50 kg is a phenotype, and having sickle-cell anemia is a phenotype.



2 **At each locus, a diploid organism possesses two alleles located on different homologous chromosomes.**

In these notes, the term characteristic or character refers to a general feature such as eye color; the term trait or phenotype refers to specific manifestations of that feature, such as blue or brown eyes. A given phenotype arises from a genotype that develops within a particular environment. The genotype determines the potential for development; it sets certain limits, or boundaries, on that development. How the phenotype develops within those limits is determined by the effects of other genes and environmental factors, and the balance between these influences varies from character to character.

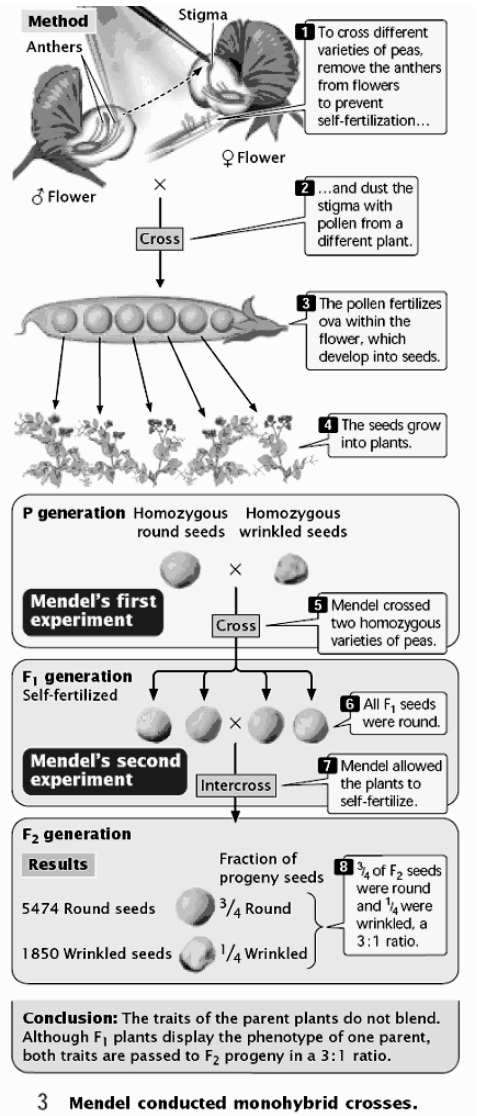
For some characters, the differences between phenotypes are determined largely by differences in genotype; in other words, the genetic limits for that phenotype are narrow. Seed shape in Mendel's peas is a good example of a characteristic for which the genetic limits are narrow and the phenotypic differences are largely genetic. For other characters, environmental differences are more important; in this case, the limits imposed by the genotype are broad. The height that an oak tree reaches at maturity is a phenotype that is strongly influenced by environmental factors, such as the availability of water, sunlight, and nutrients. Nevertheless, the tree's genotype still imposes some limits on its height: an oak tree will never grow to be 300 m tall no matter how much sunlight, water, and fertilizer are provided. Thus, even the height of an oak tree is determined to some degree by genes. For many characteristics, both genes and environment are important in determining phenotypic differences.

An obvious but important concept is that only the genotype is inherited. Although the phenotype is determined, at least to some extent, by genotype, organisms do not transmit their phenotypes to the next generation. The distinction between genotype and phenotype is one of the most important principles of modern genetics. The next section describes Mendel's careful observation of phenotypes through several generations of breeding experiments. These experiments allowed him to deduce not only the genotypes of the individual plants, but also the rules governing their inheritance.

Monohybrid Crosses

Mendel started with 34 varieties of peas and spent 2 years selecting those varieties that he would use in his experiments. He verified that each variety was genetically pure (homozygous for each of the traits that he chose to study) by growing the plants for two generations and confirming that all offspring were the same as their parents. He then carried out a number of crosses between the different varieties. Although peas are normally self-fertilizing (each plant crosses with itself), Mendel conducted crosses between different plants by opening the buds before the anthers were fully developed, removing the anthers, and then dusting the stigma with pollen from a different plant.

Mendel began by studying monohybrid crosses— those between parents that differed in a single characteristic. In one experiment, Mendel crossed a pea plant homozygous for round seeds with one that was homozygous for wrinkled seeds (FIGURE 3). This first generation of a cross is the P (parental) generation.



3 **Mendel conducted monohybrid crosses.**

After crossing the two varieties in the P generation, Mendel observed the offspring that resulted from the cross. In regard to seed characteristics, such as seed shape, the phenotype develops as soon as the seed matures, because the seed traits are determined by the newly formed embryo within the seed. For characters associated with the plant itself, such as stem length, the phenotype doesn't develop until the plant grows from the seed; for these characters, Mendel had to wait until the following spring, plant the seeds, and then observe the phenotypes on the plants that germinated.

The offspring from the parents in the P generation are the F1 (first filial) generation. When Mendel examined the F1 of this cross, he found that they expressed only one of the phenotypes present in the parental generation: all the F1 seeds were round. Mendel carried out 60 such crosses and always obtained this result. He also conducted reciprocal crosses: in one cross, pollen (the male gamete) was taken from a plant with round seeds and, in its reciprocal cross, pollen was taken from a plant with wrinkled seeds. Reciprocal crosses gave the same result: all the F1 were round. Mendel wasn't content with examining only the seeds arising from these monohybrid crosses. The following spring, he planted the F1 seeds, cultivated the plants that germinated from them, and allowed the plants to self-fertilize, producing a second generation (the F2 generation). Both of the traits from the P generation emerged in the F2; Mendel counted 5474 round seeds and 1850 wrinkled seeds in the F2 (see Figure 3.3). He noticed that the number of the round and wrinkled seeds constituted approximately a 3 to 1 ratio; that is, about of the F2 seeds were round and were wrinkled. Mendel conducted monohybrid crosses for all seven of the characteristics that he studied in pea plants, and in all of the crosses he obtained the same result: all of the F1 resembled only one of the two parents, but both parental traits emerged in the F2 in approximately a 3:1 ratio.

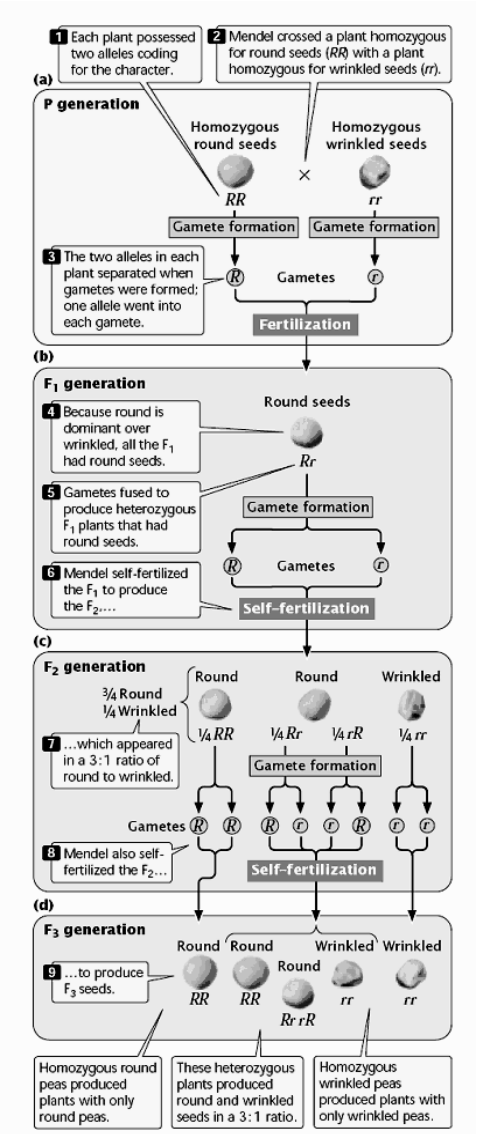
What Monohybrid Crosses Reveal

Mendel drew several important conclusions from the results of his monohybrid crosses. First, he reasoned that, although the F1 plants display the phenotype of only one parent, they must inherit genetic factors from both parents because they transmit both phenotypes to the F2 generation. The presence of both round and wrinkled seeds in the F2 could be explained only if the F1 plants possessed both round and wrinkled genetic factors that they had inherited from the P generation. He concluded that each plant must therefore possess two genetic factors coding for a character.

The genetic factors that Mendel discovered (alleles) are, by convention, designated with letters; the allele for round seeds is usually represented by R, and the allele for wrinkled seeds by r. The plants in the P generation of Mendel's cross possessed two identical alleles: RR in the round-seeded parent and rr in the wrinkled-seeded parent (FIGURE 4a).

A second conclusion that Mendel drew from his monohybrid crosses was that the two alleles in each plant separate when gametes are formed, and one allele goes into each gamete. When two gametes (one from each parent) fuse to produce a zygote, the allele from the male parent unites with the allele from the female parent to produce the genotype of the offspring. Thus, Mendel's F1 plants inherited an R allele from

the round-seeded plant and an r allele from the wrinkled-seeded plant (FIGURE 4b). However, only the trait encoded by round allele (R) was observed in the F1—all the F1 progeny had round seeds. Those traits that appeared unchanged in the F1 heterozygous offspring.



4 **Mendel's monohybrid crosses revealed the principle of segregation and the concept of dominance.**

Mendel called dominant, and those traits that disappeared in the F1 heterozygous offspring he called recessive. When dominant and recessive alleles are present together, the recessive allele is masked, or suppressed. The concept of dominance was a third important conclusion that Mendel derived from his monohybrid crosses.

Mendel's fourth conclusion was that the two alleles of an individual plant separate with equal probability into the gametes. When plants of the F1 (with genotype Rr) produced gametes, half of the gametes received the R allele for round seeds and half received the r allele for wrinkled seeds.

The gametes then paired randomly to produce the following genotypes in equal proportions among the F2: RR, Rr, rR, rr(FIGURE 4c). Because round (R) is dominant over wrinkled (r), there were three round progeny in the F2 (RR, Rr, rR) for every one wrinkled progeny (rr) in the F2. This 3:1 ratio of round to wrinkled progeny that Mendel observed in the F2 could occur only if the two alleles of a genotype separated into the gametes with equal probability.

The conclusions that Mendel developed about inheritance from his monohybrid crosses have been further developed and formalized into the principle of segregation and the concept of dominance. The principle of segregation (Mendel's first law) states that each individual diploid organism possesses two alleles for any particular characteristic. These two alleles segregate (separate) when gametes are formed, and one allele goes into each gamete. Furthermore, the two alleles segregate into gametes in equal proportions. The concept of dominance states that, when two different alleles are present in a genotype, only the trait of the dominant allele is observed in the phenotype.

Mendel confirmed these principles by allowing his F2 plants to self-fertilize and produce an F3 generation. He found that the F2 plants grown from the wrinkled seeds— those displaying the recessive trait (rr)—produced an F3 in which all plants produced wrinkled seeds. Because his wrinkled-seeded plants were homozygous for wrinkled alleles (rr) they could pass on only wrinkled alleles to their progeny (FIGURE 4d).

The F2 plants grown from round seeds—the dominant trait—fell into two types (Figure 4c). On self-fertilization, about 2/3 of the F2 plants produced both round and wrinkled seeds in the F3 generation. These F2 plants were heterozygous (Rr); so they produced ¼ RR (round), ½ Rr (round), and ¼ rr (wrinkled) seeds, giving a 3:1 ratio of round to wrinkled in the F3. About 1/3 of the F2 plants were of the second type; they produced only the dominant round-seeded trait in the F3. These F2 plants were homozygous for the round allele (RR) and thus could produce only round offspring in the F3 generation. Mendel planted the seeds obtained in the F3 and carried these plants through three more rounds of self-fertilization. In each generation, 2/3 of the round-seeded plants produced round and wrinkled offspring, whereas 1/3 produced only round offspring. These results are entirely consistent with the principle of segregation.

Relating Genetic Crosses to Meiosis

We have now seen how the results of monohybrid crosses are explained by Mendel's principle of segregation. Many students find that they enjoy working genetic crosses but are frustrated by the abstract nature of the symbols. Perhaps you feel the same at this point. You may be asking "What do these symbols really represent? What does the genotype RR mean in regard to the biology of the organism?"

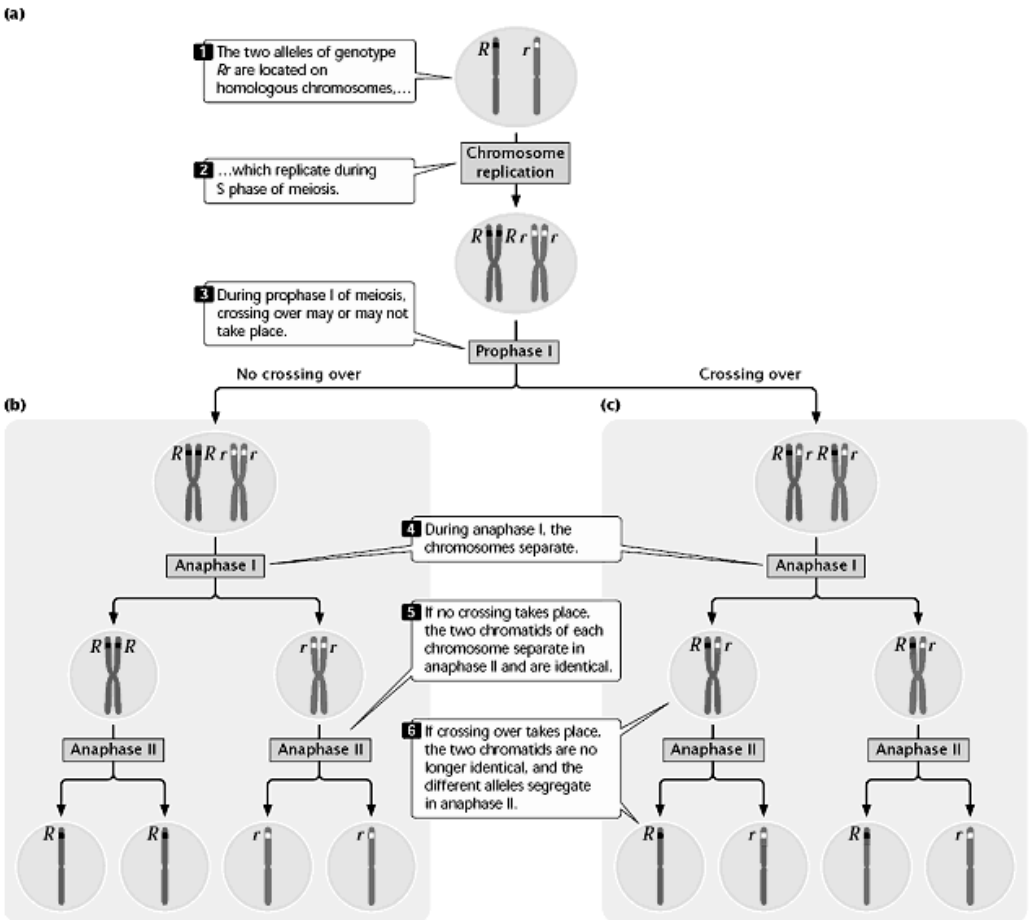
The answers to these questions lie in relating the abstract symbols of crosses to the structure and behavior of chromosomes, the repositories of genetic information.

In 1900, when Mendel's work was rediscovered and biologists began to apply his principles of heredity, the relation between genes and chromosomes was still unclear. The theory that genes are located on chromosomes (the chromosome theory of heredity) was developed in the early 1900s by Walter Sutton, then a graduate student at Columbia University. Through the careful study of meiosis in insects, Sutton documented the fact that each homologous pair of chromosomes consists of one maternal chromosome and one paternal chromosome. Showing that these pairs segregate independently into gametes in meiosis, he concluded that this process is the biological basis for Mendel's principles of heredity. The German cytologist and embryologist Theodor Boveri came to similar conclusions at about the same time.

Sutton knew that diploid cells have two sets of chromosomes. Each chromosome has a pairing partner, its homologous chromosome. One chromosome of each homologous pair is inherited from the mother and the other is inherited from the father. Similarly, diploid cells possess two alleles at each locus, and these alleles constitute the genotype for that locus. The principle of segregation indicates that one allele of the genotype is inherited from each parent.

This similarity between the number of chromosomes and the number of alleles is not accidental—the two alleles of a genotype are located on homologous chromosomes. The symbols used in genetic crosses, such as R and r, are just shorthand notations for particular sequences of DNA in the chromosomes that code for particular phenotypes. The two alleles of a genotype are found on different but homologous chromosomes. During the S stage of meiotic interphase, each chromosome replicates, producing two copies of each allele, one on each chromatid (FIGURE 5a). The homologous chromosomes segregate during anaphase I, thereby separating the two different alleles (FIGURE 5b and c). This chromosome segregation is the basis of the principle of segregation. During anaphase II of meiosis, the two chromatids of each replicated chromosome separate; so each gamete resulting from meiosis carries only a single allele at each locus, as Mendel's principle of segregation predicts.

If crossing over has taken place during prophase I of meiosis, then the two chromatids of each replicated chromosome are no longer identical, and the segregation of different alleles takes place at anaphase I and anaphase II. Of course, Mendel didn't know anything about chromosomes; he formulated his principles of heredity entirely on the basis of the results



5 **Segregation happens because homologous chromosomes separate in meiosis.**

of the crosses that he carried out. Nevertheless, we should not forget that these principles work because they are based on the behavior of actual chromosomes during meiosis.

Predicting the Outcomes of Genetic Crosses

One of Mendel's goals in conducting his experiments on pea plants was to develop a way to predict the outcome of crosses between plants with different phenotypes. In this section, we will first learn a simple, shorthand method for predicting outcomes of genetic crosses (the Punnett square), and then we will learn how to use probability to predict the results of crosses.

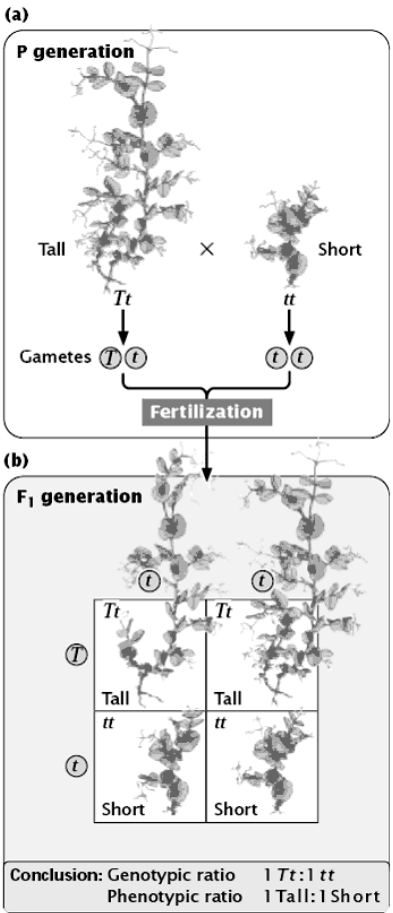
The Punnett square

To illustrate the Punnett square, let's examine another cross that Mendel carried out. By crossing two varieties of peas that differed in height, Mendel established that tall (T) was dominant over short (t). He tested his theory concerning the inheritance of dominant traits by crossing an F1 tall plant that was heterozygous (Tt) with the short homozygous parental variety (tt). This type of cross, between an F1 genotype and either of the parental genotypes, is called a backcross.

To predict the types of offspring that result from this cross, we first determine which gametes will be produced by each parent (FIGURE 6a). The principle of segregation tells us that the two alleles in each parent separate, and one allele passes to each gamete. All gametes from the homozygous tt short plant will receive a single short (t) allele. The tall plant in this cross is heterozygous (Tt); so 50% of its gametes will receive a tall allele (T) and the other 50% will receive a short allele (t).

A Punnett square is constructed by drawing a grid, putting the gametes produced by one parent along the upper edge and the gametes produced by the other parent down the left side (FIGURE 6b). Each cell (a block within the Punnett square) contains an allele from each of the corresponding gametes, generating the genotype of the progeny produced by fusion of those gametes. In the upper left-hand cell of the Punnett square in Figure 6b, a gamete containing T from the tall plant unites with a gamete containing t from the short plant, giving the genotype of the progeny (Tt). It is useful to write the phenotype expressed by each genotype; here the progeny will be tall, because the tall allele is dominant over the short allele. This process is repeated for all the cells in the Punnett square.

By simply counting, we can determine the types of progeny produced and their ratios. In Figure 6b, two cells contain tall (Tt) progeny and two cells contain short (tt) progeny; so the genotypic ratio expected for this cross is 2 Tt to 2 tt (a 1:1 ratio). Another way to express this result is to say that we expect of the progeny to have genotype Tt (and phenotype tall) and of the progeny to have genotype tt (and phenotype short). In this cross, the genotypic ratio and the phenotypic ratio are the same, but this outcome need not be the case. Try completing a Punnett square for the cross in which the F1 round-seeded plants in Figure 4 undergo self fertilization (you should obtain a phenotypic ratio of 3 round to 1 wrinkled and a genotypic ratio of 1 RR to 2 Rr to 1 rr).



6 **The Punnett square can be used for determining the results of a genetic cross.**

The Testcross

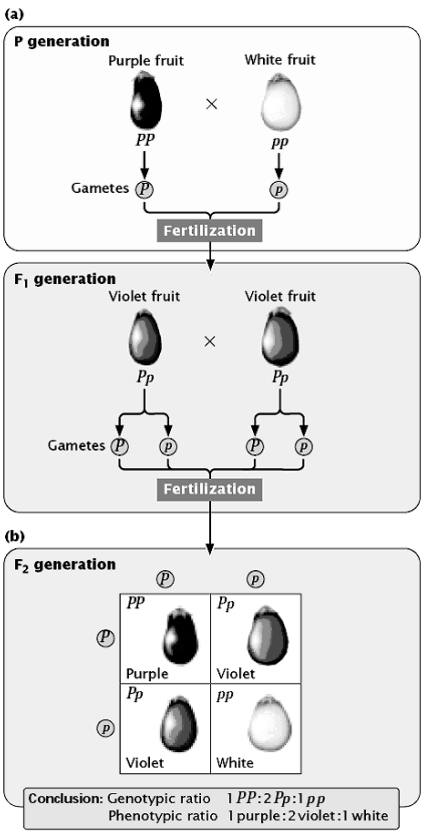
A useful tool for analyzing genetic crosses is the testcross, in which one individual of unknown genotype is crossed with another individual with a homozygous recessive genotype for the trait in question. Figure 6 illustrates a testcross (as well as a backcross). A testcross tests, or reveals, the genotype of the first individual.

Suppose you were given a tall pea plant with no information about its parents. Because tallness is a dominant trait in peas, your plant could be either homozygous (TT) or heterozygous (Tt), but you would not know which. You could determine its genotype by performing a testcross. If the plant were homozygous (TT), a testcross would produce all tall progeny (TT x tt→ all Tt); if the plant were heterozygous (Tt), the testcross would produce half tall progeny and half short progeny (Tt x tt→ ½ Tt and ½ tt). When a testcross is performed, any recessive allele in the unknown genotype is expressed in the progeny, because it will be paired with a recessive allele from the homozygous recessive parent.

Incomplete Dominance

The seven characters in pea plants that Mendel chose to study extensively all exhibited dominance, but Mendel did realize that not all characters have traits that exhibit dominance. He conducted some crosses concerning the length of time that pea plants take to flower. When he crossed two homozygous varieties that differed in their flowering time by an average of 20 days, the length of time taken by the F1 plants to flower was intermediate between those of the two parents. When the heterozygote has a phenotype intermediate between the phenotypes of the two homozygotes, the trait is said to display incomplete dominance.

Incomplete dominance is also exhibited in the fruit color of eggplants. When a homozygous plant that produces purple fruit (PP) is crossed with a homozygous plant that produces white fruit (pp), all the heterozygous F1 (Pp) produce violet fruit (FIGURE 9a). When the F1 are crossed with each other, ¼ of the F2 are purple (PP), ½ are violet (Pp), and ¼ are white (pp), as shown in FIGURE 9b. This 1:2:1 ratio is different from the 3:1 ratio that we would observe if eggplant fruit color exhibited dominance. When a trait displays incomplete dominance, the genotypic ratios and phenotypic ratios of the offspring are the same, because each genotype has its own phenotype. It is impossible to obtain eggplants that are pure breeding for violet fruit, because all plants with violet fruit are heterozygous.



9 **Fruit color in eggplant is inherited as an incompletely dominant trait.**

Another example of incomplete dominance is feather color in chickens. A cross between a homozygous black chicken and a homozygous white chicken produces F1 chickens that are gray. If these gray F1 are intercrossed, they produce F2 birds in a ratio of 1 black: 2 gray: 1 white. Leopard white spotting in horses is incompletely dominant over unspotted horses: LL horses are white with numerous dark spots, heterozygous Ll horses have fewer spots, and ll horses have no spots.

Genetic Symbols

As we have seen, genetic crosses are usually depicted with the use of symbols to designate the different alleles. Lowercase letters are traditionally used to designate recessive alleles, and uppercase letters are for dominant alleles. Two or three letters may be used for a single allele: the recessive allele for heart-shaped leaves in cucumbers is designated hl, and the recessive allele for abnormal sperm head shape in mice is designated azh. The normal allele for a character—called the wild type because it is the allele most often found in the wild—is often symbolized by one or more letters and a plus sign (+). The letter(s) chosen are usually based on the phenotype of the mutant. The first letter is lowercase if the mutant phenotype is recessive, uppercase if the mutant phenotype is dominant. For example, the recessive allele for yellow eyes in the Oriental fruit fly is represented by ye, whereas the allele for wild-type eye color is represented by ye^+. At times, the letters for the wild-type allele are dropped and the allele is represented simply by a plus sign. Superscripts and subscripts are sometimes added to distinguish between genes: Lfr1 and Lfr2 represent dominant alleles at different loci that produce lacerate leaf margins in opium poppies; El^R represents an allele in goats that restricts the length of the ears.

A slash may be used to distinguish alleles present in an individual genotype. The genotype of a goat that is heterozygous for restricted ears might be written El^+/El^R or simply +/ElR. If genotypes at more than one locus are presented together, a space may separate them. A goat heterozygous for a pair of alleles that produce restricted ears and heterozygous for another pair of alleles that produce goiter can be designated by El^+/El^R G/g.

Ratios in Simple Crosses

Now that we have had some experience with genetic crosses, let's review the ratios that appear in the progeny of simple crosses, in which a single locus is under consideration. Understanding these ratios and the parental genotypes that produce them will allow you to work simple genetic crosses quickly, without resorting to the Punnett square. Later, we will use these ratios to work more complicated crosses entailing several loci.

There are only four phenotypic ratios to understand (Table 2). The 3:1 ratio arises in a simple genetic cross when both of the parents are heterozygous for a dominant trait (Aa _ Aa). The second phenotypic ratio is the 1:2:1 ratio, which arises in the progeny of crosses between two parents heterozygous for a character that exhibits incomplete dominance (Aa x Aa). The third phenotypic ratio is the 1:1 ratio, which results from the mating of a homozygous parent and a heterozygous parent. If the character exhibits

Table 2 Phenotypic ratios for simple genetic crosses (crosses for a single locus)

Ratio	Genotypes of Parents	Genotypes of Progeny	Type of Dominance
3:1	*Aa* × *Aa*	¾ *A*__: ¼ *aa*	Dominance
1:2:1	*Aa* × *Aa*	¼ *AA*: ½ *Aa*: ¼ *aa*	Incomplete dominance
1:1	*Aa* × *aa*	½ *Aa*: ½ *aa*	Dominance or incomplete dominance
	Aa × *AA*	½ *Aa*: ½ *AA*	Incomplete dominance
Uniform progency	*AA* × *AA*	All *AA*	Dominance or incomplete dominance
	aa × *aa*	All *aa*	Dominance or incomplete dominance
	AA × *aa*	All *Aa*	Dominance or incomplete dominance
	AA × *Aa*	All *A*__	Dominance

Note: A line in a genotype, such as *A*__, indicates that any allele is possible.

dominance, the homozygous parent in this cross must carry two recessive alleles (Aa x aa) to obtain a 1:1 ratio, because a cross between a homozygous dominant parent and a heterozygous parent (AA x Aa) produces only offspring displaying the dominant trait. For a character with incomplete dominance, a 1:1 ratio results from a cross between the heterozygote and either homozygote (Aa x aa or Aa x AA).

The fourth phenotypic ratio is not really a ratio—all the offspring have the same phenotype. Several combinations of parents can produce this outcome (Table 2).

A cross between any two homozygous parents—either between two of the same homozygotes (AA x AA and aa x aa) or between two different homozygotes (AA x aa)—produces progeny all having the same phenotype. Progeny of a single phenotype can also result from a cross between a homozygous dominant parent and a heterozygote (AA x Aa). If we are interested in the ratios of genotypes instead of phenotypes, there are only three outcomes to remember (Table 3): the 1:2:1 ratio, produced by a cross between two heterozygotes; the 1:1 ratio, produced by a cross between a heterozygote and a homozygote; and the uniform progeny produced by a cross between two homozygotes.

Table 3 Genotypic ratios for simple genetic crosses (crosses for a single locus)

Ratio	Genotypes of Parents	Genotypes of Progeny
1:2:1	*Aa* × *Aa*	¼ *AA*: ½ *Aa*: ¼ *aa*
1:1	*Aa* × *aa*	½ *Aa*: ½ *aa*
	Aa × *AA*	½ *Aa*: ½ *AA*
Uniform progeny	*AA* × *AA*	All *AA*
	aa × *aa*	All *aa*
	AA × *aa*	All *Aa*

These simple phenotypic and genotypic ratios and the parental genotypes that produce them provide the key to understanding crosses for a single locus and, as you will see in the next section, for multiple loci.

Multiple-Loci Crosses

We will now extend Mendel's principle of segregation to more complex crosses for alleles at multiple loci. Understanding the nature of these crosses will require an additional principle, the principle of independent assortment.

Dihybrid Crosses

In addition to his work on monohybrid crosses, Mendel also crossed varieties of peas that differed in two characteristics (dihybrid crosses). For example, he had one homozygous variety of pea that produced round seeds and yellow endosperm; another homozygous variety produced wrinkled seeds and green endosperm. When he crossed the two, all the F1 progeny had round seeds and yellow endosperm.

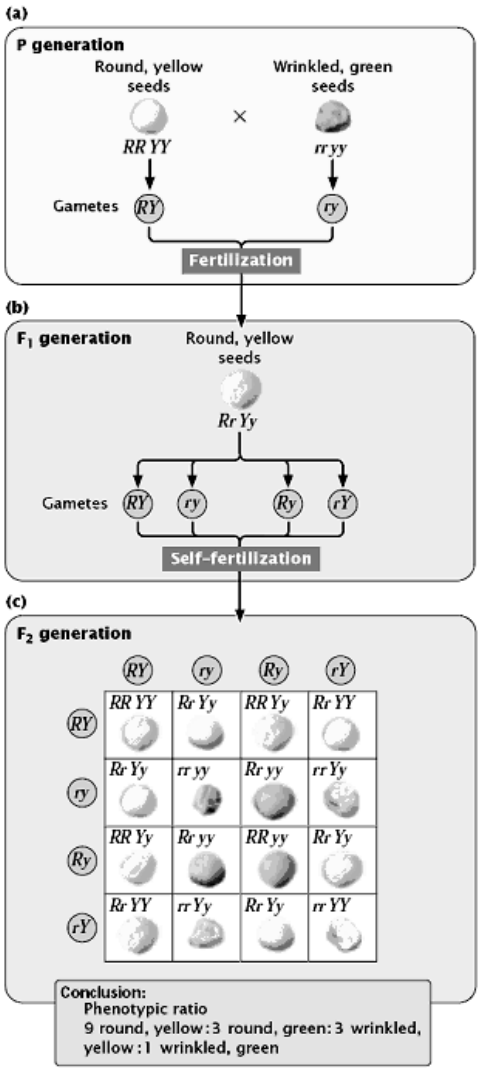
He then self-fertilized the F1 and obtained the following progeny in the F2: 315 round, yellow seeds; 101 wrinkled, yellow seeds; 108 round, green seeds; and 32 wrinkled, green seeds. Mendel recognized that these traits appeared approximately in a 9:3:3:1 ratio; that is, 9/16 of the progeny were round and yellow, 3/16 were wrinkled and yellow, 3/16 were round and green, and 1/16 were wrinkled and green.

The Principle of Independent Assortment

Mendel carried out a number of dihybrid crosses for pairs of characteristics and always obtained a 9:3:3:1 ratio in the F2. This ratio makes perfect sense in regard to segregation and dominance if we add a third principle, which Mendel recognized in his dihybrid crosses: the principle of independent assortment (Mendel's second law). This principle states that alleles at different loci separate independently of one another.

A common mistake is to think that the principle of segregation and the principle of independent assortment refer to two different processes. The principle of independent assortment is really an extension of the principle of segregation.

The principle of segregation states that the two alleles of a locus separate when gametes are formed; the principle of independent assortment states that, when these two alleles separate, their separation is independent of the separation of alleles at other loci.



11 **Mendel conducted dihybrid crosses.**

Let's see how the principle of independent assortment explains the results that Mendel obtained in his dihybrid cross. Each plant possesses two alleles coding for each characteristic, so the parental plants must have had genotypes RRYY and rryy (FIGURE 11a). The principle of segregation indicates that the alleles for each locus separate, and one allele for each locus passes to each gamete. The gametes produced by the round, yellow parent therefore contain alleles RY, whereas the gametes produced by the wrinkled, green parent contain alleles ry. These two types of gametes unite to produce the F1, all with genotype RrYy. Because round is dominant over wrinkled and yellow is dominant over green, the phenotype of the F1 will be round and yellow. When Mendel self-fertilized the F1 plants to produce the F2, the alleles for each locus separated, with one allele going into each gamete. This is where the principle of independent assortment becomes important. Each pair of alleles can separate in two ways: (1) R separates with Y and r separates with y to produce gametes RY and ry or (2) R separates with y and r separates with Y to produce gametes Ry and rY. The principle of independent assortment tells us that the alleles at each locus separate independently; thus, both kinds of separation occur equally and all four type of gametes (RY, ry, Ry, and rY) are produced in equal proportions (FIGURE 11b). When these four types of gametes are combined to produce the F2 generation, the progeny consist of 9/16 round and yellow, 3/16 wrinkled and yellow, 3/16 round and green, and 1/16 wrinkled and green, resulting in a 9:3:3:1 phenotypic ratio (FIGURE 11c).

The Relation of the Principle of Independent Assortment to Meiosis

An important qualification of the principle of independent assortment is that it applies to characters encoded by loci located on different chromosomes because, like the principle of segregation, it is based wholly on the behavior of chromosomes during meiosis. Each pair of homologous chromosomes separates independently of all other pairs in anaphase I of meiosis so genes located on different pairs of homologs will assort independently. Genes that happen to be located on the same chromosome will travel together during anaphase I of meiosis and will arrive at the same destination—within the same gamete (unless crossing over takes place). Genes located on the same chromosome therefore do not assort independently (unless they are located sufficiently far apart that crossing over takes place every meiotic division).

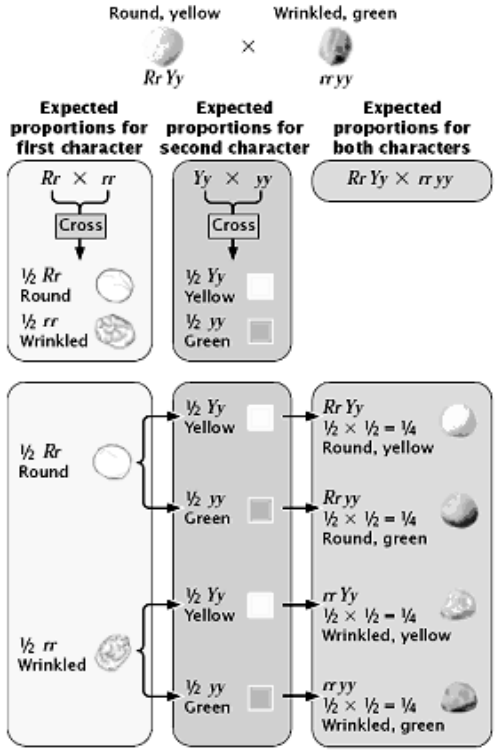
The Dihybrid Testcross

Let's practice using the branch diagram by determining the types and proportions of phenotypes in a dihybrid testcross between the round and yellow F1 plants (Rr Yy) that Mendel obtained in his dihybrid cross and the wrinkled and green plants (rryy) (FIGURE 13). Break the cross down into a series of single-locus crosses. The cross Rr x rr yields ½ round (Rr) progeny and ½ wrinkled (rr) progeny. The cross Yy x yy yields ½ yellow (Yy) progeny and ½ green (yy) progeny. Using the multiplication rule, we find the proportion of round and yellow progeny to be ½ (the probability of round) x ½ (the probability of yellow) = ¼ . Four combinations of traits with the following proportions appear in the offspring: ¼ RrYy, round yellow; ¼ Rryy, round green; rrYy, ¼ wrinkled yellow; and ¼ rryy, wrinkled green.

Trihybrid Crosses

The branch diagram can also be applied to crosses including three characters (called trihybrid crosses). In one trihybrid cross, Mendel crossed a pure-breeding variety that possessed round seeds, yellow endosperm, and gray seed coats with another pure-breeding variety that possessed wrinkled seeds, green endosperm, and white seed coats (FIGURE 14). The branch diagram shows that the expected phenotypic ratio in the F2 is 27:9:9:9:3:3:3:1, and the numbers that Mendel obtained from this cross closely fit these expected ones.

In monohybrid crosses, we have seen that three genotypes (RR, Rr, and rr) are produced in the F2. In dihybrid crosses, nine genotypes (3 genotypes for the first locus x 3 genotypes for the second locus = 9) are produced in the F2: RRYY, RRYy, RRyy, RrYY, RrYy, Rryy, rrYY, rrYy, and rryy.



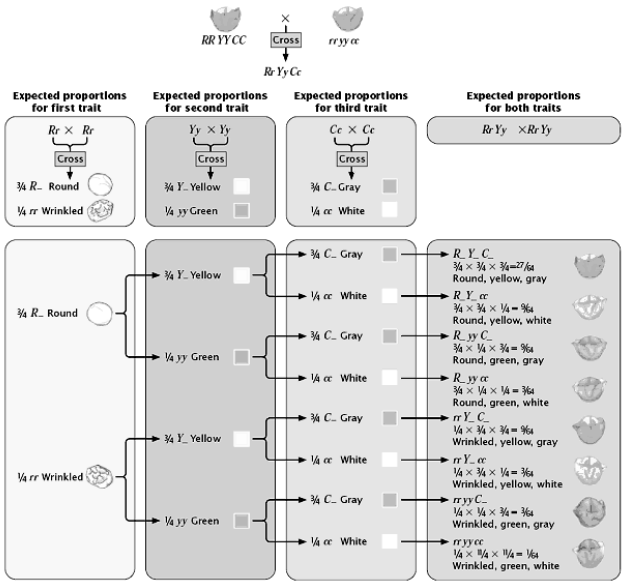
13 **A branch diagram can be used for determining the phenotypes and expected proportions of offspring from a dihybrid testcross (*RrYy* × *rryy*).**

There are three possible genotypes at each locus (when there are two alternative alleles); so the number of genotypes produced in the F2 of a cross between individuals heterozygous for n loci will be 3^n. If there is incomplete dominance, the number of phenotypes also will be 3^n because, with incomplete dominance, each genotype produces a different phenotype. If the traits exhibit dominance, the number of phenotypes will be 2^n.

Observed and Expected Ratios

When two individuals of known genotype are crossed, we expect certain ratios of genotypes and phenotypes in the progeny; these expected ratios are based on the Mendelian principles of segregation, independent assortment, and dominance. The ratios of genotypes and phenotypes actually observed among the progeny, however, may deviate from these expectations.

For example, in German cockroaches, brown body color (Y) is dominant over yellow body color (y). If we cross a brown, heterozygous cockroach (Yy) with a yellow cockroach (yy), we expect a 1:1 ratio of brown (Yy) and yellow (yy) progeny. Among 40 progeny, we would therefore expect to see 20 brown and 20 yellow offspring. However, the observed numbers might deviate from these expected values; we might in fact see 22 brown and 18 yellow progeny. Chance plays a critical role in genetic crosses, just as it does in flipping a coin. When you flip a coin, you expect a 1:1 ratio—½ heads and ½ tails. If you flip a coin 1000 times, the proportion of heads and tails obtained would probably be very close to that expected 1:1 ratio. However, if you flip the coin 10 times, the ratio of heads to tails might be quite different from 1:1. You could easily get 6 heads and 4 tails, or 3 and 7 tails, just by chance. It is possible that you might even get 10 heads and 0 tails. The same thing happens in genetic crosses. We may expect 20 brown and 20 yellow cockroaches, but 22 brown and 18 yellow progeny could arise as a result of chance.



14 **A branch diagram can be used for determining the phenotypes and expected proportions of offspring from a trihybrid cross (*RrYyCc* × *RrYyCc*).**

The Goodness-of-Fit Chi-Square Test

If you expected a 1:1 ratio of brown and yellow cockroaches but the cross produced 22 brown and 18 yellow, you probably wouldn't be too surprised even though it wasn't a perfect 1:1 ratio. In this case, it seems reasonable to assume that chance produced the deviation between the expected and the observed results. But, if you observed 25 brown and 15 yellow, would the ratio still be 1:1? Something other than chance might have caused the deviation. Perhaps the inheritance of this character is more complicated than was assumed or perhaps some of the yellow progeny died before they were counted. Clearly, we need some means of evaluating how likely it is that chance is responsible for the deviation between the observed and the expected numbers.

To evaluate the role of chance in producing deviations between observed and expected values, a statistical test called the goodness-of-fit chi-square test is used. This test provides information about how well observed values fit expected values. Before we learn how to calculate the chi square, it is important to understand what this test does and does not indicate about a genetic cross. The chi-square test cannot tell us whether a genetic cross has been correctly carried out, whether the results are correct, or whether we have chosen the correct genetic explanation for the results. What it does indicate is the probability that the difference between the observed and the expected values is due to chance. In other words, it indicates the likelihood that chance alone could produce the deviation between the expected and the observed values. If we expected 20 brown and 20 yellow progeny from a genetic cross, the chi-square

test gives the probability that we might observe 25 brown and 15 yellow progeny simply owing to chance deviations from the expected 20:20 ratio.

When the probability calculated from the chi-square test is high, we assume that chance alone produced the difference. When the probability is low, we assume that some factor other than chance—some significant factor—produced the deviation.

To use the goodness-of-fit chi-square test, we first determine the expected results. The chi-square test must always be applied to numbers of progeny, not to proportions or percentages. Let's consider a locus for coat color in domestic cats, for which black color (B) is dominant over gray (b). If we crossed two heterozygous black cats (Bb x Bb), we would expect a 3:1 ratio of black and gray kittens. A series of such crosses yields a total of 50 kittens—30 black and 20 gray. These numbers are our observed values. We can obtain the expected numbers by multiplying the expected proportions by the total number of observed progeny. In this case, the expected number of black kittens is ¾ x 50 = 37.5 and the expected number of gray kittens is ¼ x 50 = 12.5. The chi-square (X^2) value is calculated by using the following formula:

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

where Σ means the sum of all the squared differences between observed and expected divided by the expected values. To calculate the chi-square value for our black and gray kittens, we would first subtract the number of expected black kittens from the number of observed black kittens (30 - 37.5 = -7.5) and square this value: $-7.5^2 = 56.25$. We then divide this result by the expected number of black kittens, 56.25/37.5, = 1.5.We repeat the calculations on the number of expected gray kittens: $(20 - 12.5)^2/12.5 - 4.5$. To obtain the overall chi-square value, we sum the (observed - expected)^2/expected values: 1.5 + 4.5 = 6.0.

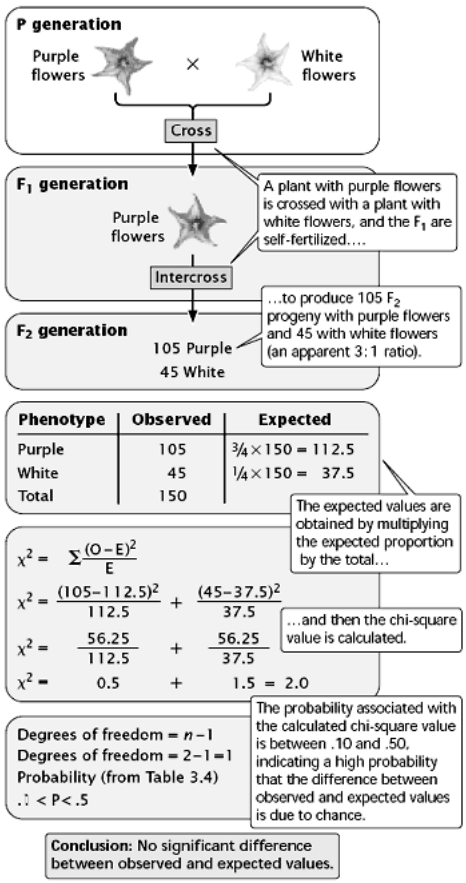
The next step is to determine the probability associated with this calculated chi-square value, which is the probability that the deviation between the observed and the expected results could be due to chance. This step requires us to compare the calculated chi-square value (6.0) with theoretical values that have the same degrees of freedom in a chi-square table. The degrees of freedom represent the number of ways in which the observed classes are free to vary. For a goodness-of-fit chi-square test, the degrees of freedom are equal to n -1, where n is the number of different expected phenotypes. In our example, there are two expected phenotypes (black and gray); so n = 2 and the degree of freedom equals 2 - 1 = 1.

Now that we have our calculated chi-square value and have figured out the associated degrees of freedom, we are ready to obtain the probability from a chi-square table (Table 4). The degrees of freedom are given in the left hand column of the table and the probabilities are given at the top; within the body of the table are chi-square values associated with these probabilities. First, find the row for the appropriate degrees of freedom; for our example with 1 degree of freedom, it is the first row of the table. Find where our calculated chi-square value (6.0) lies among the theoretical values in this row. The theoretical chi-square values increase from left to right and the probabilities decrease from left to right. Our chi-square value of 6.0 falls between the value of 5.024, associated with a probability of .025, and the value of 6.635, associated with a probability of .01.

Table 4 Critical values of the χ^2 distribution

	P								
df	.995	.975	.9	.5	.1	.05	.025	.01	.005
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801

P, probability; df, degrees of freedom.



15 **A chi-square test is used to determine the probability that the difference between observed and expected values is due to chance.**

Thus, the probability associated with our chi-square value is less than .025 and greater than .01. So, there is less than a 2.5% probability that the deviation that we observed

between the expected and the observed numbers of black and gray kittens could be due to chance. Most scientists use the .05 probability level as their cutoff value: if the probability of chance being responsible for the deviation is greater than or equal to .05, they accept that chance may be responsible for the deviation between the observed and the expected values.

When the probability is less than .05, scientists assume that chance is not responsible and a significant difference exists. The expression significant difference means that some factor other than chance is responsible for the observed values being different from the expected values. In regard to the kittens, perhaps one of the genotypes experienced increased mortality before the progeny were counted or perhaps other genetic factors skewed the observed ratios.

In choosing .05 as the cutoff value, scientists have agreed to assume that chance is responsible for the deviations between observed and expected values unless there is strong evidence to the contrary. It is important to bear in mind that even if we obtain a probability of, say, .01, there is still a 1% probability that the deviation between the observed and expected numbers is due to nothing more than chance. Calculation of the chi-square value is illustrated in (FIGURE 15).

Penetrance and Expressivity

In the genetic crosses considered thus far, we have assumed that every individual with a particular genotype expresses the expected phenotype. We assumed, for example, that the genotype Rr always produces round seeds and that the genotype rr always produces wrinkled seeds. For some characters, such an assumption is incorrect: the genotype does not always produce the expected phenotype, a phenomenon termed incomplete penetrance.

Incomplete penetrance is seen in human polydactyly, the condition of having extra fingers and toes. There are several different forms of human polydactyly, but the trait is usually caused by a dominant allele. Occasionally, people possess the allele for polydactyly (as evidenced by the fact that their children inherit the polydactyly) but nevertheless have a normal number of fingers and toes. In these cases, the gene for polydactyly is not fully penetrant.

Penetrance is defined as the percentage of individuals having a particular genotype that express the expected phenotype.

For example, if we examined 42 people having an allele for polydactyly and found that only 38 of them were polydactylous, the penetrance would be 38/42 =0.90 (90%).

A related concept is that of expressivity, the degree to which a character is expressed. In addition to incomplete penetrance, polydactyly exhibits variable expressivity. Some polydactylous persons possess extra fingers and toes that are fully functional, whereas others possess only a small tag of extra skin.

Incomplete penetrance and variable expressivity are due to the effects of other genes and to environmental factors that can alter or completely suppress the effect of a particular gene. A gene might encode an enzyme that produces a particular phenotype only within a limited temperature range.

At higher or lower temperatures, the enzyme would not function and the phenotype would not be expressed; the allele encoding such an enzyme is therefore penetrant only within a particular temperature range.

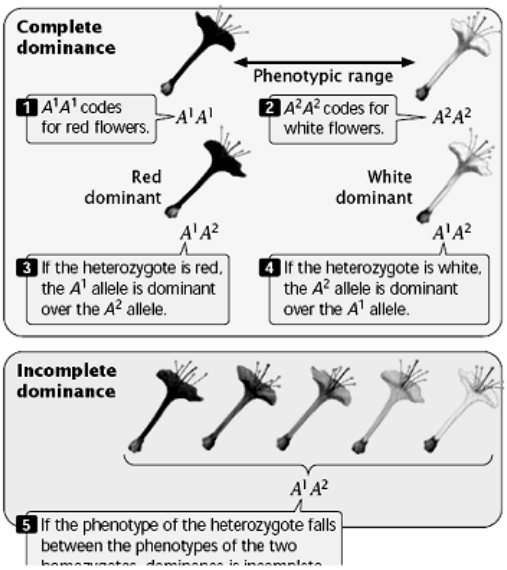
Many characters exhibit incomplete penetrance and variable expressivity, emphasizing the fact that the mere presence of a gene does not guarantee its expression.

B & C. Extensions of Mendelian principles: Codominance, incomplete dominance, gene interactions, pleiotropy, genomic imprinting, penetrance and expressivity, phenocopy, linkage and crossing over, sex linkage, sex limited and sex influenced characters. Concept of gene: Allele, multiple alleles, pseudoallele, complementation tests

Dominance Revisited

One of Mendel's important contributions to the study of heredity is the concept of dominance—the idea that an individual possesses two different alleles for a characteristic, but the trait enclosed by only one of the alleles is observed in the phenotype. With dominance, the heterozygote possesses the same phenotype as one of the homozygotes. When biologists began to apply Mendel's principles to organisms other then peas, it quickly became apparent that many characteristics do not exhibit this type of dominance. Indeed, Mendel himself was aware that dominance is not universal, because he observed that a pea plant heterozygous for long and short flowering times had a flowering time that was intermediate between those of its homozygous parents. This situation, in which the heterozygote is intermediate in phenotype between the two homozygotes, is termed incomplete dominance.

Dominance can be understood in regard to how the phenotype of the heterozygote relates to the phenotypes of the homozygotes.



2 **The type of dominance exhibited by a trait depends on how the phenotype of the heterozygote relates to the phenotypes of the homozygotes.**

In the example presented in FIGURE 2, flower color potentially ranges from red to white. One homozygous genotype, A1A1, codes for red flowers, and another, A2A2, codes for white flowers. Where the heterozygote falls on the range of phenotypes determines the type of dominance. If the heterozygote (A1A2) has flowers that are the same color as those of the A1A1 homozygote (red), then the A1 allele is completely dominant over the A2 allele; that is, red is dominant over white. If, on the other hand, the heterozygote has flowers that are the same color as the A2A2 homozygote (white), then the A2 allele is completely dominant, and white is dominant over red.When the heterozygote falls in between the phenotypes of the two homozygotes, dominance is incomplete. With incomplete dominance, the heterozygote need not be exactly intermediate (pink in our example) between the two homozygotes; it might be a slightly lighter shade of red or a slightly pink shade of white. As long as the heterozygote's phenotype can be differentiated and falls within the range of the two homozygotes, dominance is incomplete. The important thing to remember about dominance is that it affects the phenotype that genes produce, but not the way in which genes are inherited.

Another type of interaction between alleles is codominance, in which the phenotype of the heterozygote is not intermediate between the phenotypes of the homozygotes; rather, the heterozygote simultaneously expresses the phenotypes of both homozygotes. An example of codominance is seen in the MN blood types.

The MN locus codes for one of the types of antigens on red blood cells. Unlike antigens foreign to the ABO and Rh blood groups (which also code for red-blood-cell antigens), foreign MN antigens do not elicit a strong immunological reaction, and therefore the MN blood types are not routinely considered in blood transfusions. At the MN locus, there are two alleles: the L^M allele, which codes for the M antigen; and the L^N allele, which codes for the N antigen. Homozygotes with genotype L^ML^M express the M antigen on their red blood cells and have the M blood type. Homozygotes with genotype L^NL^N express the N antigen and have the N blood type. Heterozygotes with genotype L^ML^N exhibit codominance and express both the M and the N antigens; they have blood type MN. The differences between dominance, incomplete dominance, and codominance are summarized in Table 1.

Table 1 Differences between dominance, incomplete dominance, and codominance

Type of Dominance	Definition
Dominance	Phenotype of the heterozygote is the same as the phenotype of one of the homozygotes
Incomplete dominance	Phenotype of the heterozygote is intermediate (falls within the range) between the phenotypes of the two homozygotes
Codominance	Phenotype of the heterozygote includes the phenotypes of both homozygotes

The type of dominance that a character exhibits frequently depends on the level of the phenotype examined.

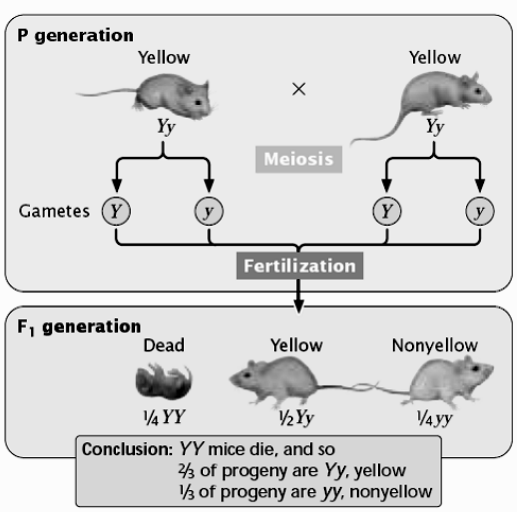
An example is cystic fibrosis, one of the more common genetic disorders found in Caucasians and usually considered to be a recessive disease. People who have cystic fibrosis produce large quantities of thick, sticky mucus, which plugs up the airways of the lungs and clogs the ducts leading from the pancreas to the intestine, causing frequent respiratory infections and digestive problems. Even with medical treatment, patients with cystic fibrosis suffer chronic, life threatening medical problems.

The gene responsible for cystic fibrosis resides on the long arm of chromosome 7. It encodes a protein termed cystic fibrosis transmembrane conductance regulator, mercifully abbreviated CFTR, which acts as a gate in the cell membrane and regulates the movement of chloride ions into and out of the cell. Patients with cystic fibrosis have a mutated, dysfunctional form of CFTR that causes the channel to stay closed, and so chloride ions build up in the cell. This buildup causes the formation of thick mucus and produces the symptoms of the disease. Most people have two copies of the normal allele for CFTR, and produce only functional CFTR protein. Those with cystic fibrosis possess two copies of the mutated CFTR allele, and produce only the defective CFTR protein. Heterozygotes, with one normal and one defective CFTR allele, produce both functional and defective CFTR protein. Thus, at the molecular level, the alleles for normal and defective CFTR are codominant, because both alleles are expressed in the heterozygote. However, because one normal allele produces enough functional CFTR protein to allow normal chloride transport, the heterozygote exhibits no adverse effects, and the mutated CFTR allele appears to be recessive at the physiological level.

In summary, several important characteristics of dominance should be emphasized. First, dominance is a result of interactions between genes at the same locus; in other words, dominance is allelic interaction. Second, dominance does not alter the way in which the genes are inherited; it only influences the way in which they are expressed as a phenotype. The allelic interaction that characterizes dominance is therefore interaction between the products of the genes. Finally, dominance is frequently "in the eye of the beholder," meaning that the classification of dominance depends on the level at which the phenotype is examined. As we saw with cystic fibrosis, an allele may exhibit codominance at one level and be recessive at another level.

Lethal Alleles

In 1905, Lucien Cuenot reported a peculiar pattern of inheritance in mice. When he mated two yellow mice, approximately of their 2/3 offspring were yellow and ¼ were non yellow. When he test-crossed the yellow mice, he found that all were heterozygous; he was never able to obtain a yellow mouse that bred true. There was a great deal of discussion about Cuenot's results among his colleagues, but it was eventually realized that the yellow allele must be lethal when homozygous (FIGURE 3). A lethal allele is one that causes death at an early stage of development— often before birth—and so a some genetypes may not appear among the progeny.



3 A 2 : 1 ratio among the progeny of a cross results from the segregation of a lethal allele.

Cuenot originally crossed two mice heterozygous for yellow: Yy x Yy. Normally, this cross would be expected to produce ¼ YY, ½ Yy, and ¼ yy (see Figure 3). The homozygous YY mice are conceived but never complete development, which leaves a 2 : 1 ratio of Yy (yellow) to yy (nonyellow) in the observed offspring; all yellow mice are heterozygous (Yy).

Another example of a lethal allele, originally described by Erwin Baur in 1907, is found in snapdragons. The aurea strain in these plants has yellow leaves. When two plants with yellow leaves are crossed, 2/3 of the progeny have yellow leaves and 1/3 have green leaves. When green is crossed with green, all the progeny have green leaves; however, when yellow is crossed with green, ½ of the progeny are green and ½ are yellow, confirming that all yellow-leaved snapdragons are heterozygous. A 2 : 1 ratio is almost always produced by a recessive lethal allele; so observing this ratio among the progeny of a cross between individuals with the same phenotype is a strong clue that one of the alleles is lethal.

In both of these examples, the lethal alleles are recessive because they cause death only in homozygotes. Unlike its effect on survival, the effect of the allele on color is dominant; in both mice and snapdragons, a single copy of the allele in the heterozygote produces a yellow color. Lethal alleles also can be dominant; in this case, homozygotes and heterozygotes for the allele die. Truly dominant lethal alleles cannot be transmitted unless they are expressed after the onset of reproduction, as in Huntington disease.

Multiple Alleles

Most of the genetic systems that we have examined so far consist of two alleles. In Mendel's peas, for instance, one allele coded for round seeds and another for wrinkled seeds; in cats, one allele produced a black coat and another produced a gray coat. For some loci, more than two alleles are present within a group of individuals—the locus has multiple alleles. (Multiple alleles may also be referred to as an allelic series.) Although there may be more than two alleles present within a group, the genotype of each diploid

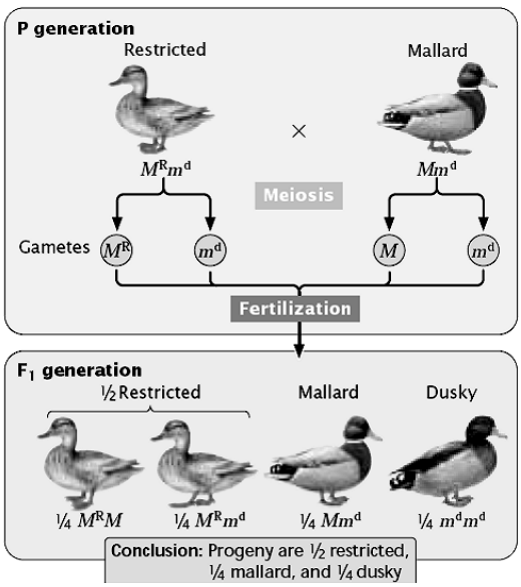
individual still consists of only two alleles. The inheritance of characteristics encoded by multiple alleles is no different from the inheritance of characteristics encoded by two alleles, except that a greater variety of genotypes and phenotypes are possible.

Duck-Feather Patterns

An example of multiple alleles is seen at a locus that determines the feather pattern of mallard ducks. One allele, M, produces the wild-type mallard pattern. A second allele, M^R, produces a different pattern called restricted, and a third allele, m^d, produces a pattern termed dusky. In this allelic series, restricted is dominant over mallard and dusky, and mallard is dominant over dusky: $M^R > M > m^d$. The six genotypes possible with these three alleles and their resulting phenotypes are:

Genotype	Phenotype
M^RM^R	restricted
M^RM	restricted
M^Rm^d	restricted
MM	mallard
Mm^d	mallard
m^dm^d	dusky

In general, the number of genotypes possible will be [n(n-1)]/2, where n equals the number of different alleles at a locus. Working crosses with multiple alleles is no different from working crosses with two alleles; Mendel's principle of segregation still holds, as shown in the cross between a restricted duck and a mallard duck (FIGURE 4).

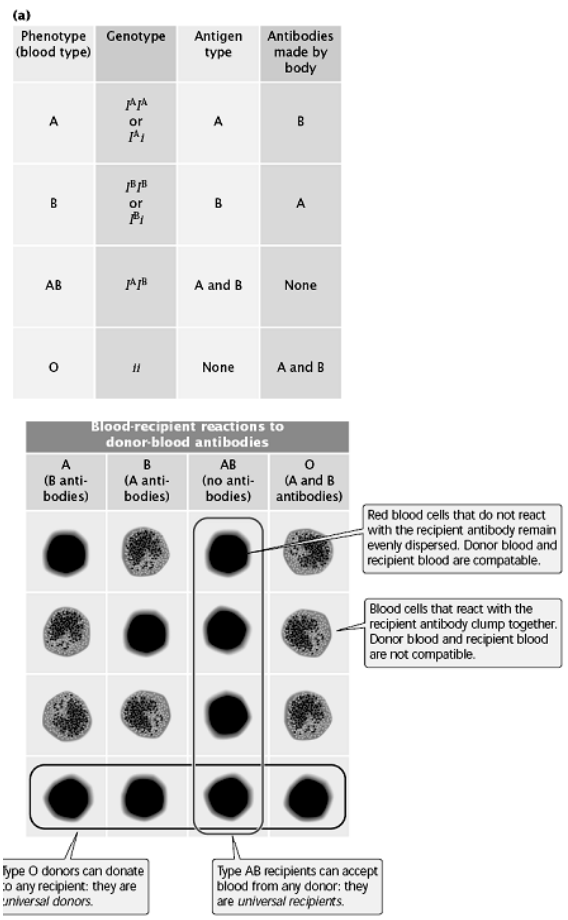


4 **Mendel's principle of segregation applies to crosses with multiple alleles.** In this example, three alleles determine the type of plumage in mallard ducks: M^R (Restricted) > M (Mallard) > m^d (Dusky).

The ABO Blood Group

Another multiple-allele system is at the locus for the ABO blood group. This locus determines your ABO blood type and, like the MN locus, codes for antigens on red blood cells. The three common alleles for the ABO blood group locus are: I^A, which codes for the A antigen; I^B, which codes for the B antigen; and i, which codes for no antigen (O).We can represent the dominance relations among the ABO alleles as follows: $I^A > i$, $I^B > i$, $I^A = I^B$. The I^A and I^B alleles are both dominant over i and are codominant with each other; the AB phenotype is due to the presence of an I^A allele and an IB allele, which results in the production of A and B antigens on red blood cells. An individual with genotype ii produces neither antigen and has blood type O. The six common genotypes at this locus and their phenotypes are shown in FIGURE 5a.

Antibodies are produced against any foreign antigens (see Figure 5a). For instance, a person having blood type A produces B antibodies, because the B antigen is foreign. A person having blood type B produces A antibodies, and someone having blood type AB produces neither A nor B antibodies, because neither A nor B antigen is foreign. A person having blood type O possesses no A or B antigens; consequently that person produces both A antibodies and B antibodies. The presence of antibodies against foreign ABO antigens means that successful blood transfusions are possible only between persons with certain compatible blood types (FIGURE 5b).



5 **ABO blood types and possible blood transfusions.**

The inheritance of alleles at the ABO locus can be illustrated by a paternity suit involving the famous movie actor Charlie Chaplin. In 1941, Chaplin met a young actress named Joan Barry, with whom he had an affair. The affair ended in February 1942 but, 20 months later, Barry gave birth to a baby girl and claimed that Chaplin was the father. Barry then sued for child support. At this time, blood typing had just come into widespread use, and Chaplin's attorneys had Chaplin, Barry, and the child blood typed. Barry had blood type A, her child had blood type B, and Chaplin had blood type O. Could Chaplin have been the father of Barry's child?

Your answer should be no. Joan Barry had blood type A, which can be produced by either genotype I^AI^A or I^Ai. Her baby possessed blood type B, which can be produced by either genotype I^BI^B or I^Bi. The baby could not have inherited the I^B allele from Barry (Barry could not carry an IB allele if she were blood type A); therefore the baby must have inherited the i allele from her. Barry must have had genotype I^Ai, and the baby must have had genotype I^Bi Because the baby girl inherited her i allele from Barry, she must have inherited the I^B allele from her father. With blood type O, produced only by genotype ii, Chaplin could not have been the father of Barry's child. In the course of the trial to settle the paternity suit, three pathologists came to the witness stand and declared that it was genetically impossible for Chaplin to have fathered the child. Nevertheless, the jury ruled that Chaplin was the father and ordered him to pay child support and Barry's legal expenses.

Gene Interaction

In the dihybrid crosses that we examined, each locus had an independent effect on the phenotype. When Mendel crossed a homozygous round and yellow plant (RRYY) with a homozygous wrinkled and green plant (rryy)and then self-fertilized the F1, he obtained F2 progeny in the following proportions:

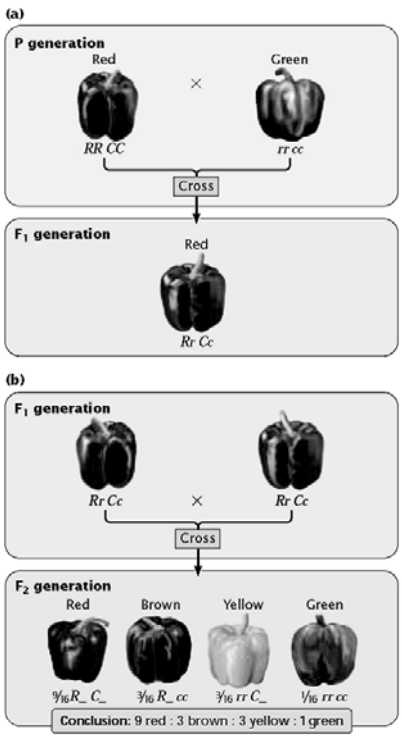
9/16	*R_Y_*	round, yellow
3/16	*R_yy*	round, green
3/16	*rrY_*	wrinkled, yellow
1/16	*rryy*	wrinkled, green

In this example, the genes showed two kinds of independence. First, the genes at each locus are independent in their assortment in meiosis, which is what produces the 9:3:3:1 ratio of phenotypes in the progeny, in accord with Mendel's principle of independent assortment. Second, the genes are independent in their phenotypic expression; the R and r alleles affect only the shape of the seed and have no influence on the color of the endosperm; the Y and y alleles affect only color and have no influence on the shape of the seed.

Frequently, genes exhibit independent assortment but do not act independently in their phenotypic expression; instead, the effects of genes at one locus depend on the presence of genes at other loci. This type of interaction between the effects of genes at different loci (genes that are not allelic) is termed gene interaction. With gene interaction, the products of genes at different loci combine to produce new phenotypes that are not predictable from the single-locus effects alone. In our consideration of gene interaction, we'll focus primarily on interaction between the effects of genes at two loci, although interactions among genes at three, four, or more loci are common.

Gene Interaction That Produces Novel Phenotypes

Let's first examine gene interaction in which genes at two loci interact to produce a single characteristic. Fruit color in the pepper Capsicum annuum is determined in this way. This plant produces peppers in one of four colors: red, brown, yellow, or green. If a homozygous plant with red peppers is crossed with a homozygous plant with green peppers, all the F1 plants have red peppers (FIGURE 6a). When the F1 are crossed with one another, the F2 are in a ratio of 9 red : 3 brown : 3 yellow : 1 green (FIGURE 6b).



6 **Gene interaction in which two loci determine a single characteristic, fruit color, in the pepper *Capsicum annuum*.**

This dihybrid ratio is produced by a cross between two plants that are both heterozygous for two loci (RrCc X RrCc). In peppers, a dominant allele R at the first locus produces a red pigment; the recessive allele r at this locus produces no red pigment. A dominant allele C at the second locus causes decomposition of the green pigment chlorophyll; the recessive allele c allows chlorophyll to persist. The genes at the two loci then interact to produce the colors seen in F2 peppers:

Genotype	Phenotype
*R_C_*	red
R_cc	brown
*rrC_*	yellow
rrcc	green

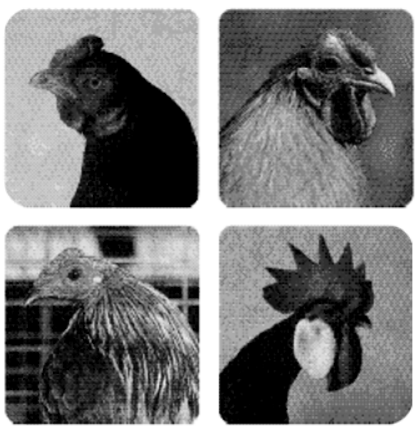
To illustrate how Mendel's rules of heredity can be used to understand the inheritance of characteristics determined by

gene interaction, let's consider a testcross between an F1 plant from the cross in Figure 6 (RrCc) and a plant with green peppers (rrcc). For independent loci, we can work this cross by breaking it down into two simple crosses. At the first locus, the heterozygote Rr is crossed with the homozygote rr; this cross produces ½ Rr and ½ rr progeny. Similarly, at the second locus, the heterozygous genotype Cc is crossed with the homozygous genotype cc, producing ½ Cc and ½ cc progeny. In accord with Mendel's principle of independent assortment, these single-locus ratios can be combined by using the multiplication rule: the probability of obtaining the genotype RrCc is the probability of Rr (1/2) multiplied by the probability of Cc (1/2), or ¼ . The probability of each progeny genotype resulting from the testcross is:

Progeny genotype	Probability at each locus	Overall probability	Phenotype
RrCc	$1/2 \times 1/2 =$	$1/4$	red peppers
Rrcc	$1/2 \times 1/2 =$	$1/4$	brown peppers
rrCc	$1/2 \times 1/2 =$	$1/4$	yellow peppers
rrcc	$1/2 \times 1/2 =$	$1/4$	green peppers

When you work problems with gene interaction, it is especially important to determine the probabilities of single locus genotypes and to multiply the probabilities of genotypes, not phenotypes, because the phenotypes cannot be determined without considering the effects of the genotypes at all the contributing loci.

Another example of gene interaction that produces novel phenotypes is seen in the genes that determine comb shape in chickens. The comb is the fleshy structure found on the head of a chicken. Genes at two loci (R, r and P, p) interact to determine the four types of combs shown in FIGURE 7.



7 **A chicken's comb is determined by gene interaction between two loci.** (a) A walnut comb is produced when there is a dominant allele at each of two loci (*R_P_*). (b) A rose comb occurs when there is a dominant allele only at the first locus (*R_pp*). (c) A pea comb occurs when there is a dominant allele only at the second locus (*ppR_*). (d) A single comb is produced by the presence of only recessive alleles at both loci (*rrpp*).

A walnut comb is produced when at least one dominant allele R is present at the first locus and at least one dominant allele P is present at the second locus (genotype R_P_). A chicken with at least one dominant allele at the first locus and two recessive alleles at the second locus (genotype R_pp) possesses a rose comb. If two recessive alleles are present at the first locus and at least one dominant allele is present at the second (genotype rrP_), the chicken has a pea comb. Finally, if two recessive alleles are present at both loci (rrpp), the bird has a single comb.

Gene Interaction with Epistasis

Sometimes the effect of gene interaction is that one gene masks (hides) the effect of another gene at a different locus, a phenomenon known as epistasis. This phenomenon is similar to dominance, except that dominance entails the masking of genes at the same locus (allelic genes). In epistasis, the gene that does the masking is called the epistatic gene; the gene whose effect is masked is a hypostatic gene. Epistatic genes may be recessive or dominant in their effects.

Recessive epistasis

Recessive epistasis is seen in the genes that determine coat color in Labrador retrievers. These dogs may be black, brown, or yellow; their different coat colors are determined by interactions between genes at two loci (although a number of other loci also help to determine coat color). One locus determines the type of pigment produced by the skin cells: a dominant allele B codes for black pigment, whereas a recessive allele b codes for brown pigment. Alleles at a second locus affect the deposition of the pigment in the shaft of the hair; allele E allows dark pigment (black or brown) to be deposited, whereas a recessive allele e prevents the deposition of dark pigment, causing the hair to be yellow. The presence of genotype ee at the second locus therefore masks the expression of the black and brown alleles at the first locus. The genotypes that determine coat color and their phenotypes are:

Genotype	Phenotype
*B_ E_*	black
*bbE_*	brown (frequently called chocolate)
B_ee	yellow
bbee	yellow

If we cross a black Labrador homozygous for the dominant alleles with a yellow Labrador homozygous for the recessive alleles and then intercross the F1, we obtain progeny in the F2 in a 9 : 3 : 4 ratio:

P *BBEE* (black) × *bbee* (yellow)

↓

F_1 *BbEe* (black)

↓ Intercross

F_2 $^9/_{16}$ *B_E_* black; $^3/_{16}$ *bbE_* brown; $^3/_{16}$ *B_ee* yellow; $^1/_{16}$ *bbee* yellow } $^4/_{16}$ yellow

Notice that yellow dogs can carry alleles for either black or brown pigment, but these alleles are not expressed in their coat color.

In this example of gene interaction, allele e is epistatic to B and b, because e masks the expression of the alleles for black and brown pigments, and alleles B and b are hypostatic to e. In this case, e is a recessive epistatic allele, because two copies of e must be present to mask of the black and brown pigments.

Dominant epistasis

Dominant epistasis is seen in the interaction of two loci that determine fruit color in summer squash, which is commonly found in one of three colors: yellow, white, or green.When a homozygous plant that produces white squash is crossed with a homozygous plant that produces green squash and the F1 plants are crossed with each other, the following results are obtained:

P plants with white squash × plants with green squash

↓

F_1 plants with white squash

↓ Intercross

F_2 $^{12}/_{16}$ plants with white squash
$^{3}/_{16}$ plants with yellow squash
$^{1}/_{16}$ plants with green squash

How can gene interaction explain these results? In the F2, 12/16 or ¾ of the plants produce white squash and 3/16+1/16 =4/16 =1/4 of the plants produce squash having color. This outcome is the familiar 3 : 1 ratio produced by a cross between two heterozygous individuals, which suggests that a dominant allele at one locus inhibits the production of pigment, resulting in white progeny. If we use the symbol W to represent the dominant allele that inhibits pigment production, then genotype W_ inhibits pigment production and produces white squash, whereas ww allows pigment and results in colored squash.

Among those ww F2 plants with pigmented fruit, we observe 3/16 yellow and 1/16 green (a 3 : 1 ratio). This outcome is because a second locus determines the type of pigment produced in the squash, with yellow (Y_) dominant over green (yy). This locus is expressed only in ww plants, which lack the dominant inhibitory allele W.We can assign the genotype wwY_ to plants that produce yellow squash and the genotype wwyy to plants that produce green squash.

The genotypes and their associated phenotypes are:

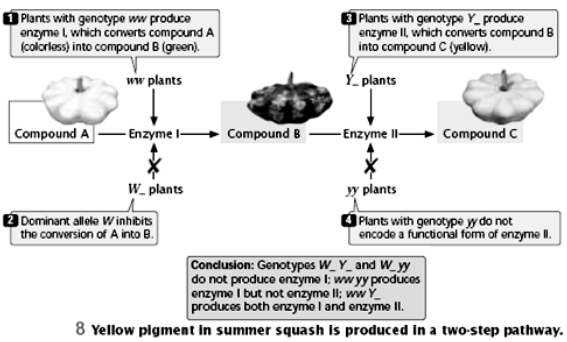
*W_Y_*	white squash
W_yy	white squash
*wwY_*	yellow squash
wwyy	green squash

Allele W is epistatic to Y and y—it suppresses the expression of these pigment-producing genes.W is a dominant epistatic allele because, in contrast with e in Labrador retriever coat color, a single copy of the allele is sufficient to inhibit pigment production.

Summer squash provides us with a good opportunity for considering how epistasis often arises when genes affect a series of steps in a biochemical pathway. Yellow pigment in the squash is most likely produced in a two-step biochemical pathway (FIGURE 8). A colorless (white) compound (designated A in Figure 8) is converted by enzyme I into green compound B, which is then converted into compound C by enzyme II. Compound C is the yellow pigment in the fruit.

Plants with the genotype ww produce enzyme I and may be green or yellow, depending on whether enzyme II is present. When allele Y is present at a second locus, enzyme II is produced and compound B is converted into compound C, producing a yellow fruit. When two copies of y, which does not encode a functional form of enzyme II, are present, squash remain green. The presence of W at the first locus inhibits the conversion of compound A into compound B; plants with genotype W_ do not make compound B and their fruit remains white, regardless of which alleles are present at the second locus.

Many cases of epistasis arise in this way. A gene (such as W) that has an effect on an early step in a biochemical pathway will be epistatic to genes (such as Y and y) that affect subsequent steps, because the effect of the enzyme in the later step depends on the product of the earlier reaction.



8 **Yellow pigment in summer squash is produced in a two-step pathway.**

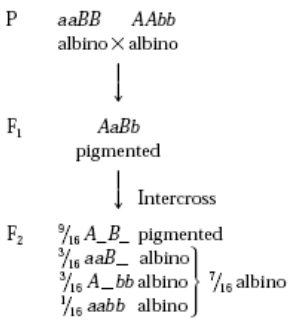
Duplicate recessive epistasis

(COMPLEMENATARY GENES)

Let's consider one more detailed example of epistasis. Albinism is the absence of pigment and is a common genetic trait in many plants and animals. Pigment is almost always produced through a multistep biochemical pathway; thus, albinism may entail gene interaction. Robert T. Dillon and Amy R. Wethington found that albinism in the common freshwater snail Physa heterostroha can result from the presence of either of two recessive alleles at two different loci. Inseminated snails were collected from a natural population and placed in cups of water, where they laid eggs. Some of the eggs hatched into albino snails. When two albino snails were crossed, all of the F1 were pigmented. On intercrossing the F1, the F2 9/16 consisted of pigmented snails and 7/16 albino snails. How did this 9 :7 ratio arise?

The 9 :7 ratio seen in the F2 snails can be understood as a modification of the 9:3:3:1 ratio obtained when two individuals heterozygous for two loci are crossed. The 9:7 ratio arises when dominant alleles at both loci (A_B_)

produce pigmented snails; any other genotype produces albino snails:



The 9:7 ratio in these snails is probably produced by a two step pathway of pigment production. Pigment (compound C) is produced only after compound A has been converted into compound B by enzyme I and after compound B has been converted into compound C by enzyme II. At least one dominant allele A at the first locus is required to produce enzyme I; similarly, at least one dominant allele B at the second locus is required to produce enzyme II. Albinism arises from the absence of compound C, which may happen in three ways. First, two recessive alleles at the first locus (genotype aaB_) may prevent the production of enzyme I, and so compound B is never produced. Second, two recessive alleles at the second locus (genotype A_bb) may prevent the production of enzyme II.

In this case, compound B is never converted into compound C. Third, two recessive alleles may be present at both loci (aabb), causing the absence of both enzyme I and enzyme II. In this example of gene interaction, a is epistatic to B, and b is epistatic to A; both are recessive epistatic alleles because the presence of two copies of either allele a or b is necessary to suppress pigment production. This example differs from the suppression of coat color in Labrador retrievers in that recessive alleles at either of two loci are capable of suppressing pigment production in the snails, whereas recessive alleles at a single locus suppress pigment expression in Labs.

Interpreting Ratios Produced by Gene Interaction

A number of modified ratios that result from gene interaction are shown in Table 5.2. Each of these examples represents a modification of the basic 9:3:3:1 dihybrid ratio. In interpreting the genetic basis of modified ratios, we should keep several points in mind. First, the inheritance of the genes producing these characteristics is no different from the inheritance of genes coding for simple genetic characters. Mendel's principles of segregation and independent assortment still apply; each individual possesses two alleles at each locus, which separate in meiosis, and genes at the different loci assort independently. The only difference is in how the products of the genotypes interact to produce the phenotype. Thus, we cannot consider the expression of genes at each locus separately, but must take into consideration how the genes at different loci interact.

A second point is that in the examples that we have considered, the phenotypic proportions were always in sixteenths because, in all the crosses, pairs of alleles segregated at two independently assorting loci. The probability of inheriting one of the two alleles at a locus is ½. Because there are two loci, each with two alleles, the probability of inheriting any particular combination of genes is $(½)^4 = 1/16$. For a trihybrid cross, the progeny proportions should be in sixty-fourths, because $(1/2)^6 = 1/64$. In general, the progeny proportions should be in fractions of $(1/2)^{2n}$, where n equals the number of loci with two alleles segregating in the cross.

Table 2 Modified dihybrid—phenotypic ratios due to gene interaction

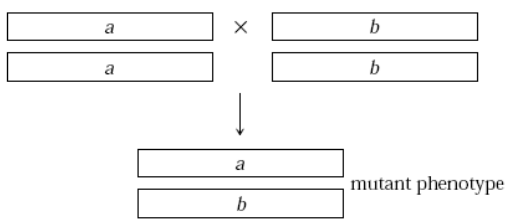
Ratio	Genotype A_B_	A_bb	aaB_	aabb	Type of Interaction	Example
9:3:3:1	9	3	3	1	None	Seed shape and endosperm color in peas
9:3:4	9	3	4		Recessive epistasis	Coat color in Labrador retrievers
12:3:1	12		3	1	Dominant epistasis	Color in squash
9:7	9		7		Duplicate recessive epistasis	Albinism in snails
9:6:1	9	6		1	Duplicate interaction	—
15:1	15			1	Duplicate dominant epistasis	—
13:3	13		3		Dominant and recessive epistasis	—

[a]Reading across, each row gives the phenotypic ratios of progeny from a dihybrid cross (*AaBb* × *AaBb*).

Complementation: Determining Whether Mutations Are at the Same or Different Loci

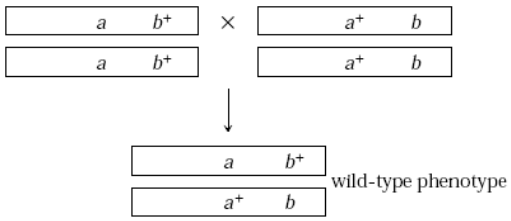
How do we know whether different mutations that affect a characteristic occur at the same locus (are allelic) or at different loci? In fruit flies, for example, white is an X-linked mutation that produces white eyes instead of the red eyes found in wild-type flies. Apricot is an X-linked recessive mutation that produces light orange-colored eyes. Do the white and apricot mutations occur at the same locus or at different loci? We can use the complementation test to answer this question.

To carry out a complementation test, parents that are homozygous for different mutations are crossed, producing offspring that are heterozygous. If the mutations are allelic (occur at the same locus), then the heterozygous offspring have only mutant alleles (ab) and exhibit a mutant phenotype:



If, on the other hand, the mutations occur at different loci, each of the homozygous parents possesses wild-type genes at the other locus (aa b^+ b^+ and a^+ a^+ bb); so the heterozygous

offspring inherit a mutant and a wild-type allele at each locus. In this case, the mutations complement each other and the heterozygous offspring have the wild-type phenotype:



Complementation occurs when an individual possessing two mutant genes has a wild-type phenotype and is an indicator that the mutations are non-allelic genes. When the complementation test is applied to white and apricot mutations, all of the heterozygous offspring have light colored eyes, demonstrating that white and apricot are produced by mutations that occur at the same locus and are allelic.

Interaction Between Sex and Heredity

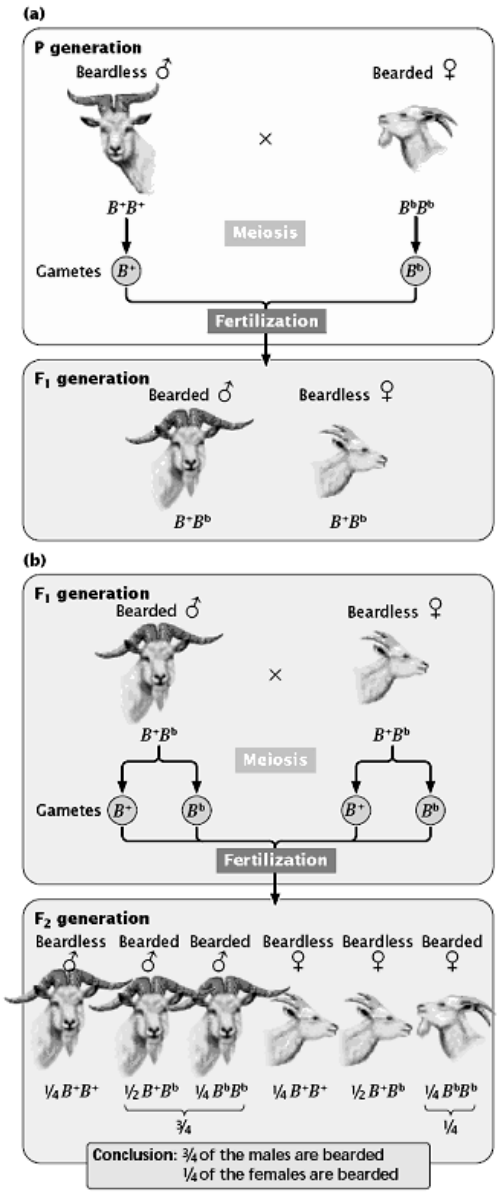
In previous section, we considered characteristics encoded by genes located on the sex chromosomes and how their inheritance differs from the inheritance of traits encoded by autosomal genes. Now we will examine additional influences of sex, including the effect of the sex of an individual on the expression of genes on autosomal chromosomes, characteristics determined by genes located in the cytoplasm, and characteristics for which the genotype of only the maternal parent determines the phenotype of the offspring. Finally, we'll look at situations in which the expression of genes on autosomal chromosomes is affected by the sex of the parent from whom they are inherited.

Sex-Influenced and Sex-Limited Characteristics

Sex influenced characteristics are determined by autosomal genes and are inherited according to Mendel's principles, but they are expressed differently in males and females.

In this case, a particular trait is more readily expressed in one sex; in other words, the trait has higher penetrance in one of the sexes. For example, the presence of a beard on some goats is determined by an autosomal gene (B^b) that is dominant in males and recessive in females. In males, a single allele is required for the expression of this trait: both the homozygote (B^bB^b) and the heterozygote (B^bB^+) have beards, whereas the B^+B^+ male is beardless. In contrast, females require two alleles in order for this trait to be expressed: the homozygote B^bB^b has a beard, whereas the heterozygote (B^bB^+) and the other homozygote (B^+B^+) are beardless. The key to understanding the expression of the bearded gene is to look at the heterozygote.

In males (for which the presence of a beard is dominant), the heterozygous genotype produces a beard but, in females (for which the presence of a beard is recessive and its absence is dominant), the heterozygous genotype produces a goat without a beard.



9 Genes that encode sex-influenced traits are inherited according to Mendel's principles but are expressed differently in males and females.

FIGURE 9a illustrates a cross between a beardless male (B_B_) and a bearded female (B^bB^b). The alleles separate into gametes according to Mendel's principle of segregation, and all the F1 are heterozygous (B_B^b). Because the trait is dominant in males and recessive in females, all the F1 males will be bearded, and all the F1 females will be beardless. When the F1 are crossed with one another, ¼ of the F2 progeny are B^bB^b, ½ are B^bB_, and are ¼ B_B_ (FIGURE 9b). Because male heterozygotes are bearded, ¾ of the males in the F2 possess beards; because female heterozygotes are beardless, only ¼ of the females in F2 are bearded.

An example of a sex-influenced characteristic in humans is pattern baldness, in which hair is lost prematurely from the front and the top of the head. Pattern baldness is an

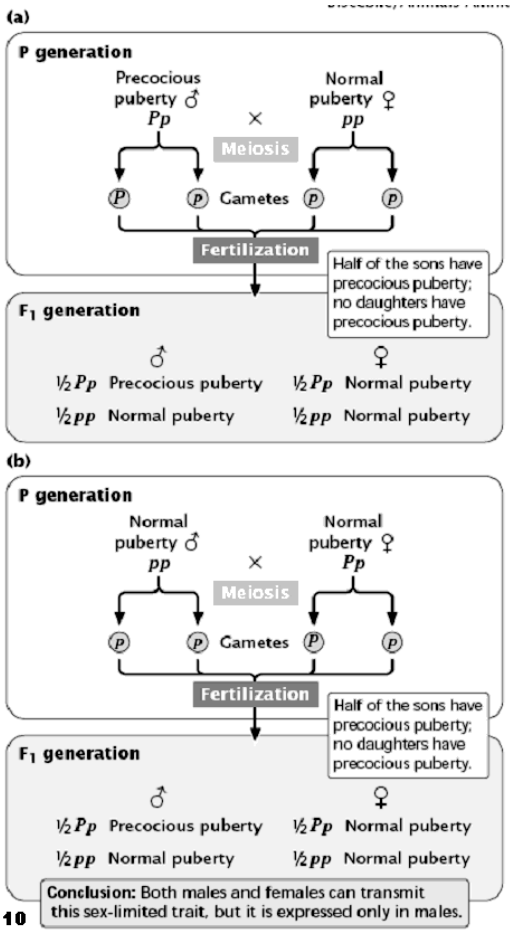
autosomal character believed to be dominant in males and recessive in females, just like beards in goats. Contrary to a popular misconception, a man does not inherit pattern baldness from his mother's side of the family (which would be the case if the character were X linked, but it isn't). Pattern baldness is autosomal; men and women can inherit baldness from either their mothers or their fathers. Men require only a single allele for baldness to become bald, whereas women require two alleles for baldness, and so pattern baldness is much more common among men. Furthermore, pattern baldness is expressed weakly in women; those with the trait usually have only a mild thinning of the hair, whereas men frequently lose all the hair on the top of the head. The expression of the allele for pattern baldness is clearly enhanced by the presence of male sex hormones; males who are castrated at an early age rarely become bald (but castration is not a recommended method for preventing baldness).

An extreme form of sex-influenced inheritance, a sex limited characteristic is encoded by autosomal genes that are expressed in only one sex—the trait has zero penetrance in the other sex. In domestic chickens, some males display a plumage pattern called cock feathering. Other males and all females display a pattern called hen feathering. Cock feathering is an autosomal recessive trait that is sex limited to males. Because the trait is autosomal, the genotypes of males and females are the same, but the phenotypes produced by these genotypes differ in males and females:

Genotype	Male phenotype	Female phenotype
HH	hen feathering	hen feathering
Hh	hen feathering	hen feathering
hh	cock feathering	hen feathering

An example of a sex-limited characteristic in humans is male-limited precocious puberty. There are several types of precocious puberty in humans, most of which are not genetic. Male-limited precocious puberty, however, results from an autosomal dominant allele (P) that is expressed only in males; females with the gene are normal in phenotype.

Males with precocious puberty undergo puberty at an early age, usually before the age of 4. At this time, the penis enlarges, the voice deepens, and pubic hair develops. There is no impairment of sexual function; affected males are fully fertile. Most are short as adults, because the long bones stop growing after puberty. Because the trait is rare, affected males are usually heterozygous (Pp). A male with precocious puberty who mates with a woman who has no family history of this condition will transmit the allele for precocious puberty to ½ of the children (FIGURE 10a), but it will be expressed only in the sons. If one of the heterozygous daughters (Pp) mates with a male who has normal puberty (pp), ½ of the sons will exhibit precocious puberty (FIGURE 10b). Thus a sex-limited characteristic can be inherited from either parent, although the trait appears in only one sex.



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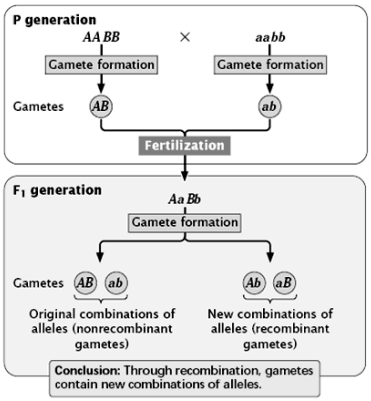
The results of molecular studies reveal that the underlying genetic defect in male-limited precocious puberty affects the receptor for luteinizing hormone (LH). This hormone normally attaches to receptors found on certain cells of the testes and stimulates these cells to produce testosterone. During normal puberty in males, high levels of LH stimulate the increased production of testosterone, which, in turn, stimulates the anatomical and physiological changes associated with puberty. The P allele for precocious puberty codes for a defective LH receptor, which stimulates testosterone production even in the absence of LH. Boys with this allele produce high levels of testosterone at an early age, when levels of LH are low. Defective LH receptors are also found in females who carry the precocious-puberty gene, but their presence does not result in precocious puberty, because additional hormones are required along with LH to induce puberty in girls.

D. Gene mapping methods: Linkage maps, tetrad analysis, mapping with molecular markers, mapping by using somatic cell hybrids, development of mapping population in plants.

Linkage and Independent Assortment

We have introduced Mendel's principles of segregation and independent assortment. Let's take a moment to review these two important concepts. The principle of segregation states that each diploid individual possesses two alleles that separate in meiosis, with one allele going into each gamete.

The principle of independent assortment provides additional information about the process of segregation: it tells us that the two alleles separate independently of alleles at other loci. The independent separation of alleles produces recombination, the sorting of alleles into new combinations. Consider a cross between individuals homozygous for two different pairs of alleles: AABB X aabb. The first parent, AABB, produces gametes with alleles AB, and the second parent, aabb, produces gametes with the alleles ab, resulting in F1 progeny with genotype AaBb (FIGURE 1). Recombination means that, when one of the F1 progeny reproduces, the combination of alleles in its gametes may differ from the combinations in the gametes of its parents. In other words, the F1 may produce gametes with alleles Ab or aB in addition to gametes with AB or ab.



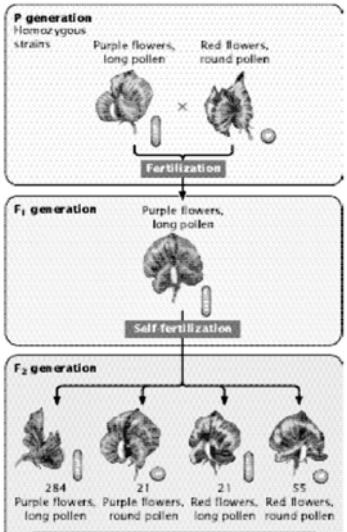
1 Recombination is the sorting of alleles into new combinations.

Mendel derived his principles of segregation and independent assortment by observing progeny of genetic crosses, but he had no idea of what biological processes produced these phenomena. In 1903, Walter Sutton proposed a biological basis for Mendel's principles, called the chromosome theory of heredity. This theory holds that genes are found on chromosomes. Let's restate Mendel's two principles in terms of the chromosome theory of heredity.

The principle of segregation states that each diploid individual possesses two alleles for a trait, each of which is located at the same position, or locus, on each of the two homologous chromosomes. These chromosomes segregate in meiosis, with each gamete receiving one homolog. The principle of independent assortment states that, in meiosis, each pair of homologous chromosomes assorts independently of other homologous pairs. With this new perspective, it is easy to see that the number of chromosomes in most organisms is limited and that there are certain to be more genes than chromosomes; so some genes must be present on the same chromosome and should not assort independently.

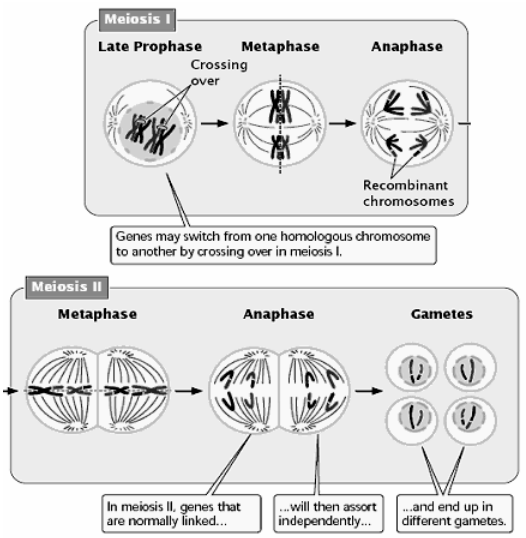
Genes located close together on the same chromosome are called linked genes and belong to the same linkage group. As we've said, linked genes travel together during meiosis, eventually arriving at the same destination (the same gamete), and are not expected to assort independently. However, all of the characteristics examined by Mendel in peas did display independent assortment and, after the rediscovery of Mendel's work, the first genetic characteristics studied in other organisms also seemed to assort independently. How could genes be carried on a limited number of chromosomes and yet assort independently?

The apparent inconsistency between the principle of independent assortment and the chromosome theory of heredity soon disappeared, as biologists began finding genetic characteristics that did not assort independently. One of the first cases was reported in sweet peas by William Bateson, Edith Rebecca Saunders, and Reginald C. Punnett in 1905. They crossed a homozygous strain of peas having purple flowers and long pollen grains with a homozygous strain having red flowers and round pollen grains. All the F1 had purple flowers and long pollen grains, indicating that purple was dominant over red and long was dominant over round.When they intercrossed the F1, the resulting F2 progeny did not appear in the 9:3:3:1 ratio expected with independent assortment (FIGURE 2). An excess of F2 plants had purple flowers and long pollen or red flowers and round pollen (the parental phenotypes). Although Bateson, Saunders, and Punnett were unable to explain these results, we now know that the two loci that they examined lie close together on the same chromosome and therefore do not assort independently.



Linkage and Recombination Between Two Genes

Genes on the same chromosome are like passengers on a charter bus: they travel together and ultimately arrive at the same destination. However, genes occasionally switch from one homologous chromosome to another through the process of crossing over (FIGURE 3). Crossing over produces recombination—it breaks up the associations of genes imposed by linkage. As will be discussed later, genes located on the same chromosome can exhibit independent assortment if they are far enough apart. In summary, linkage adds a further complication to interpretations of the results of genetic crosses. With an understanding of how linkage affects heredity, we can analyze crosses for linked genes and successfully predict the types of progeny that will be produced.



3 Crossing over takes place in meiosis and is responsible for recombination.

Notation for Crosses with Linkage

In analyzing crosses with linked genes, we must know not only the genotypes of the individuals crossed, but also the arrangement of the genes on the chromosomes. To keep track of this arrangement, we will introduce a new system of notation for presenting crosses with linked genes. Consider a cross between an individual homozygous for dominant alleles at two linked loci and another individual homozygous for recessive alleles at those loci. Previously, we would have written these genotypes as: AABB x aabb

For linked genes, however, it's necessary to write out the specific alleles as they are arranged on each of the homologous chromosomes:

$$\frac{A \quad B}{A \quad B} \times \frac{a \quad b}{a \quad b}$$

In this notation, each line represents one of the two homologous chromosomes. In the first parent of the cross, each homologous chromosome contains A and B alleles; in the second parent, each homologous chromosome contains a and b alleles. Inheriting one chromosome from each parent, the F1 progeny will have the following genotype:

$$\frac{A \quad B}{a \quad b}$$

Here, the importance of designating the alleles on each chromosome is clear. One chromosome has the two dominant alleles A and B, whereas the homologous chromosome has the two recessive alleles a and b. The notation can be simplified by drawing only a single line, with the understanding that genes located on the same side of the line lie on the same chromosome:

$$\frac{A \quad B}{a \quad b}$$

This notation can be simplified further by separating the alleles on each chromosome with a slash: AB/ab. Remember that the two alleles at a locus are always located on different homologous chromosomes and therefore must lie on opposite sides of the line. Consequently, we would never write the genotypes as:

$$\frac{A \quad a}{B \quad b}$$

because the alleles A and a can never be on the same chromosome.

It is also important to always keep the same order of the genes on both sides of the line; thus, we should never write:

$$\frac{A \quad B}{b \quad a}$$

because this would imply that alleles A and b are allelic (at the same locus).

Complete Linkage Compared with Independent Assortment

We will first consider what happens to genes that exhibit complete linkage, meaning that they are located on the same chromosome and do not exhibit detectable crossing over. Genes rarely exhibit complete linkage but, without the complication of crossing over, the effect of linkage can be seen more clearly. We will then consider what happens when genes assort independently. Finally, we will consider the results obtained if the genes are linked but exhibit some crossing over.

A testcross reveals the effects of linkage. For example, if a heterozygous individual is test-crossed with a homozygous recessive individual (AaBb X aabb), whatever alleles are present in the gametes contributed by the heterozygous parent will be expressed in the phenotype of the offspring, because the homozygous parent could not contribute dominant alleles that might mask them. Consequently, traits that appear in the progeny reveal which alleles were transmitted by the heterozygous parent.

Consider a pair of linked genes in tomato plants. One pair affects the type of leaf: an allele for mottled leaves (m) is recessive to an allele that produces normal leaves (M). Nearby on the same chromosome is another locus that determines the height of the plant: an allele for dwarf (d) is recessive to an allele for tall (D). Testing for linkage can be done with a testcross, which requires a plant heterozygous for both traits. A geneticist might produce this heterozygous plant by crossing a variety of tomato that is homozygous for normal leaves and tall height with a variety that is homozygous for mottled leaves and dwarf height:

$$\text{P}\quad \frac{M}{M}\,\frac{D}{D} \times \frac{m}{m}\,\frac{d}{d}$$

$$\downarrow$$

$$\text{F}_1\quad \frac{M}{m}\,\frac{D}{d}$$

The geneticist would then use these F1 heterozygotes in a testcross, crossing them with plants homozygous for mottled leaves and dwarf height:

$$\frac{M}{m}\,\frac{D}{d} \times \frac{m}{m}\,\frac{d}{d}$$

The results of this testcross are diagrammed in FIGURE 4a. During gamete formation, the heterozygote produces two types of gametes: some with the M D chromosome and others with the m d chromosome. Because no crossing over occurs, these gametes are the only types produced by the heterozygote. Notice that these gametes contain only combinations of alleles that were present in the original parents: either the allele for normal leaves together with the allele for tall height (M and D) or the allele for mottled leaves together with the allele for dwarf height (m and d). Gametes that contain only original combinations of alleles present in the parents are non recombinant gametes, or parental gametes.

The homozygous parent in the testcross produces only one type of gamete; it contains chromosome m d and pairs with one of the two gametes generated by the heterozygous parent (see Figure 4a). Two types of progeny result: half have normal leaves and are tall:

$$\frac{M}{m}\,\frac{D}{d}$$

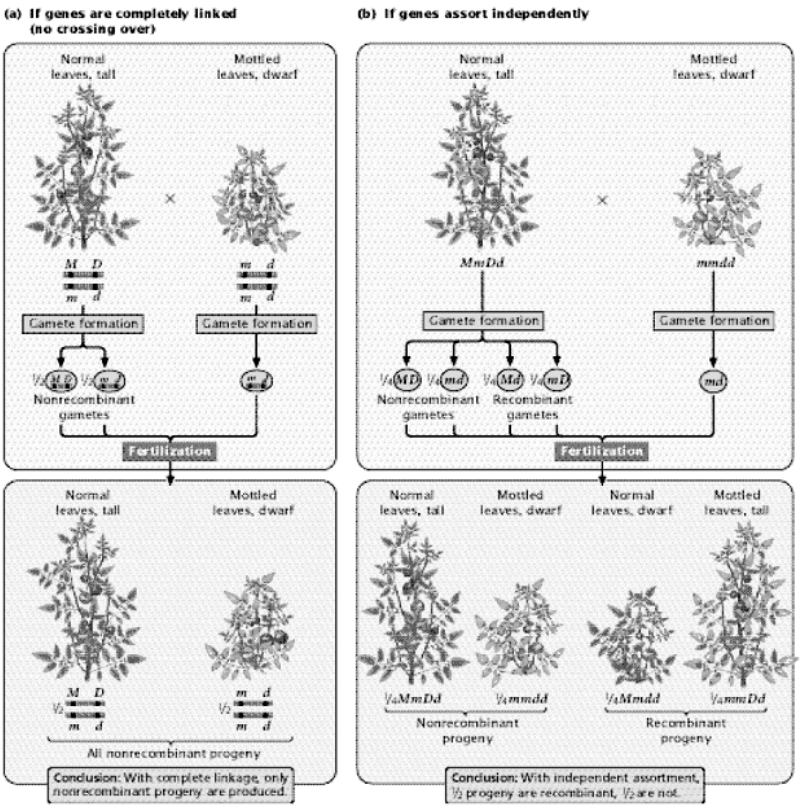
and half have mottled leaves and are dwarf:

$$\frac{m}{m}\,\frac{d}{d}$$

These progeny display the original combinations of traits present in the P generation and are non recombinant progeny, or parental progeny. No new combinations of the two traits, such as normal leaves with dwarf or mottled leaves with tall, appear in the offspring, because the genes affecting the two characteristics are completely linked and are inherited together. New combinations of traits could arise only if the linkage between M and D or between m and d were broken.

These results are distinctly different from the results that are expected when genes assort independently (FIGURE 4b). With independent assortment, the heterozygous plant (MmDd) would produce four types of gametes: two nonrecombinant gametes containing the original combinations of alleles (MD and md) and two gametes containing new combinations of alleles (Md and mD). Gametes with new combinations of alleles are called recombinant gametes. With independent assortment, nonrecombinant and recombinant gametes are produced in equal proportions.

These four types of gametes join with the single type of gamete produced by the homozygous parent of the testcross to produce four kinds of progeny in equal proportions (see Figure 4b). The progeny with new combinations of traits formed from recombinant gametes are termed recombinant progeny.

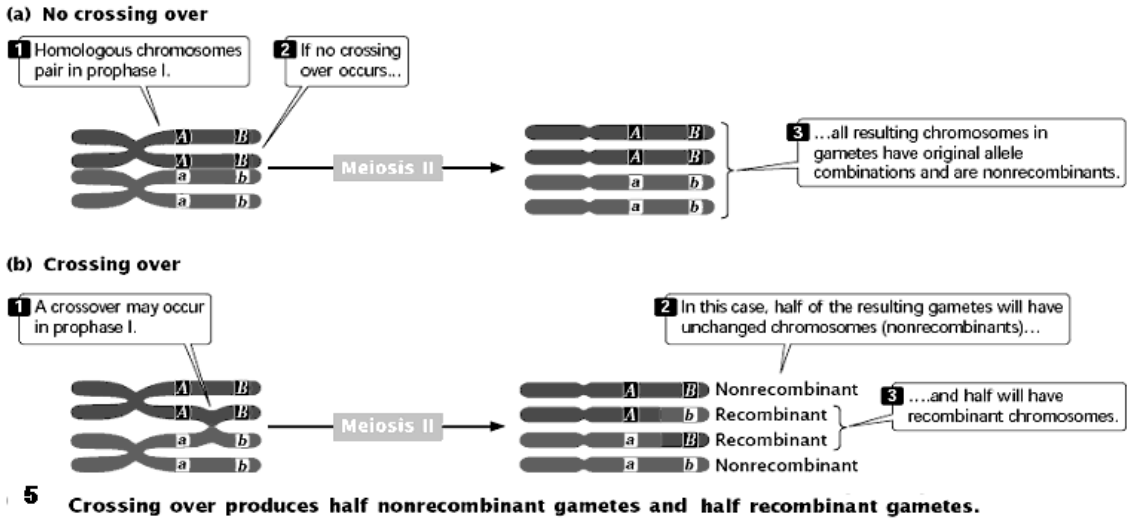


4 A testcross reveals the effects of linkage. Results of a testcross for two loci in tomatoes that determine leaf type and plant height.

In summary, a testcross in which one of the plants is heterozygous for two completely linked genes yields two types of progeny, each type displaying one of the original combinations of traits present in the P generation. Independent assortment, in contrast, produces two types of recombinant progeny and two types of non recombinant progeny in equal proportions.

Crossing Over with Linked Genes

Linkage is rarely complete—usually, there is some crossing over between linked genes (incomple linkage), producing new combinations of traits. Let's see how this occurs. Theory The effect of crossing over on the inheritance of two linked genes is shown in FIGURE 5. Crossing over, which takes place in prophase I of meiosis, is the exchange of genetic material between nonsister chromatids. After a single crossover has taken place, the two chromatids that did not participate in crossing over are unchanged; gametes that receive these chromatids are nonrecombinants. The other two chromatids, which did participate in crossing over, now contain new combinations of alleles; gametes that receive these chromatids are recombinants.



5 Crossing over produces half nonrecombinant gametes and half recombinant gametes.

For each meiosis in which a single crossover takes place, then, two nonrecombinant gametes and two recombinant gametes will be produced. This result is the same as that produced by independent assortment; so, when crossing over between two loci takes place in every meiosis, it is impossible to determine whether the genes were linked and crossing over took place or whether the genes are on different chromosomes.

For closely linked genes, crossing over does not take place in every meiosis. In meioses in which there is no crossing over, only non recombinant gametes are produced. In meioses in which there is a single crossover, half the gametes are recombinants and half are non recombinants (because a single crossover only affects two of the four chromatids); so the total percentage of recombinant gametes is always half the percentage of meioses in which crossing over takes place.

Even if crossing over between two genes takes place in every meiosis, only 50% of the resulting gametes will be recombinants. Thus, the frequency of recombinant gametes is always half the frequency of crossing over, and the maximum proportion of recombinant gametes is 50%.

Application

Let us apply what we have learned about linkage and recombination to a cross between tomato plants that differ in the genes that code for leaf type and plant height. Assume now that these genes are linked and that some crossing over takes place between them. Suppose a geneticist carried out the testcross outlined earlier:

$$\frac{M}{m}\ \frac{D}{d} \times \frac{m}{m}\ \frac{d}{d}$$

When crossing over takes place between the genes for leave type and height, two of the four gametes produced will be recombinants. When there is no crossing over, all four resulting gametes will be nonrecombinants. Thus, over all, the majority of gametes will be nonrecombinants. These gametes then unite with gametes produced by the homozygous recessive parent, which contain only the recessive alleles, resulting in mostly nonrecombinant progeny and a few recombinant progeny (FIGURE 6). In this cross, we see that 55 of the testcross progeny have normal leaves and are tall and 53 have mottled leaves and are dwarf. These plants are the nonrecombinant progeny, containing the original combinations of traits that were present in the parents. Of the 123 progeny, 15 have new combinations of traits that were not seen in the parents: 8 are normal leaved and dwarf, and 7 are mottle leaved and tall. These plants are the recombinant progeny. The results of a cross such as the one illustrated in Figure 6 reveal several things. A testcross for two independently assorting genes is expected to produce a 1:1:1:1 phenotypic ratio in the progeny. The progeny of this cross clearly do not exhibit such a ratio; so we might suspect that the genes are not assorting independently. When linked genes undergo crossing over, the result is mostly nonrecombinant progeny and fewer recombinant progeny. This result is what we observe among the progeny of the testcross illustrated in Figure 6; so we conclude that two genes show evidence of linkage with some crossing over.

Calculation of Recombination Frequency

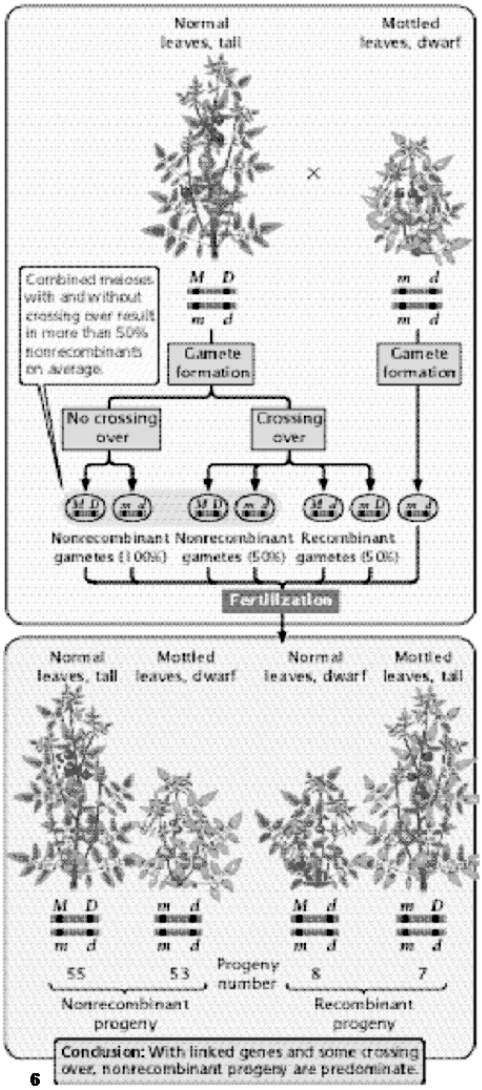
The percentage of recombinant progeny produced in a cross is called the recombination frequency, which is calculated as follows:

$$\text{recombinant frequency} = \frac{\text{number of recombinant progeny}}{\text{total number of progeny}} \times 100\%$$

In the testcross shown in Figure 7, 15 progeny exhibit new combinations of traits; so the recombination frequency is:

$$\frac{8+7}{55+53+8+7} \times 100\% = \frac{15}{123} \times 100\% = 12\%$$

Thus, 12% of the progeny exhibit new combinations of traits resulting from crossing over.



6

Coupling and Repulsion

In crosses for linked genes, the arrangement of alleles on the homologous chromosomes is critically important in determining the outcome of the cross. For example, consider the inheritance of two linked genes in the Australian blowfly, Lucilia cuprina. In this species, one locus determines the color of the thorax: purple thorax (p) is recessive to the normal green thorax (p^+). A second locus determines the color of the puparium: a black puparium (b) is recessive to the normal brown puparium (b^+). These loci are located close together on the second chromosome. Suppose we test-cross a fly that is heterozygous at both loci with a fly that is homozygous recessive at both. Because these genes are linked, there are two possible arrangements on the chromosomes of the heterozygous fly. The dominant alleles for green thorax (p^+) and brown puparium (b^+) might reside on the same chromosome, and the recessive alleles for purple thorax (p) and black puparium (b) might reside on the other homologous chromosome:

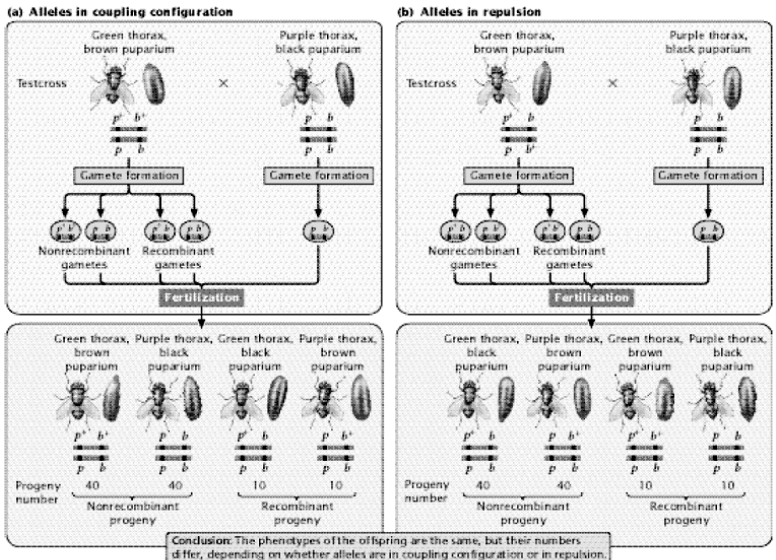
$$\frac{p^+ \quad b^+}{p \quad b}$$

This arrangement, in which wild-type alleles are found on one chromosome and mutant alleles are found on the other chromosome, is referred to as coupling, or the cis configuration. Alternatively, one chromosome might bear the alleles for green thorax (p^+) and black puparium (b), and the other chromosome would carry the alleles for purple thorax (p) and brown puparium (b^+):

$$\frac{p^+ \quad b}{p \quad b^+}$$

This arrangement, in which each chromosome contains one wild-type and one mutant allele, is called the repulsion or trans configuration. Whether the alleles in the heterozygous parent are in coupling or repulsion determines which phenotypes will be most common among the progeny of a testcross.

When the alleles are in the coupling configuration, the most numerous progeny types are those with green thorax and brown puparium and those with purple thorax and black puparium (FIGURE 7a); but, when the alleles of the heterozygous parent are in repulsion, the most numerous progeny types are those with green thorax and black puparium and those with purple thorax and brown puparium (FIGURE 7b). Notice that the genotypes of the parents in Figure 7a and b are the same (p^+ p b^+ b x pp bb) and that the dramatic difference in the phenotypic ratios of the progeny in the two crosses results entirely from the configuration— coupling or repulsion—of the



chromosomes. It is essential to know the arrangement of the alleles on the chromosomes to accurately predict the outcome of crosses in which genes are linked.

Relating Independent Assortment, Linkage, and Crossing Over

We have now considered three situations concerning genes at different loci. First, the genes may be located on different chromosomes; in this case, they exhibit independent assortment and combine randomly when gametes are formed. An individual heterozygous at two loci (AaBb) produces four types of gametes (AB, ab, Ab, and aB) in equal proportions: two types of non-recombinants and two types of recombinants.

Second, the genes may be completely linked—meaning that they're on the same chromosome and lie so close together that crossing over between them is rare. In this case, the genes do not recombine. An individual heterozygous for two closely linked genes in the coupling configuration:

$$\frac{A \quad B}{a \quad b}$$

produces only the non recombinant gametes containing alleles AB or ab. The alleles do not assort into new combinations such as Ab or aB. The third situation, incomplete linkage, is intermediate between the two extremes of independent assortment and complete linkage. Here, the genes are physically linked on the same chromosome, which prevents independent assortment. However, occasional crossovers break up the linkage and allow them to recombine. With incomplete linkage, an individual heterozygous at two loci produces four types of gametes—two types of recombinants and two types of non recombinants—but the non recombinants are produced more frequently than the recombinants because crossing over does not take place in every meiosis.

Linkage and crossing over are two opposing forces: linkage binds alleles at different loci together, restricting their ability to associate freely, whereas crossing over breaks the linkage and allows alleles to assort into new combinations. Earlier, the term recombination was defined as the sorting of alleles into new combinations. We can now distinguish between two types of recombination that differ in the mechanism that generates these new combinations of alleles.

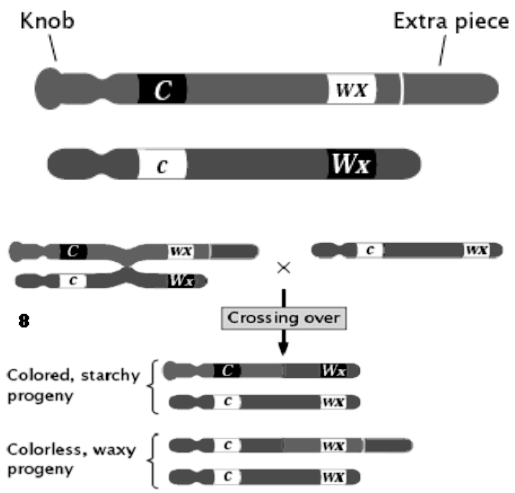
Inter-chromosomal recombination is between genes on different chromosomes. It arises from independent assortment—the random segregation of chromosomes in anaphase I of meiosis. Intra-chromosomal recombination is between genes located on the same chromosome. It arises from crossing over—the exchange of genetic material in prophase I of meiosis. Both types of recombination produce new allele combinations in the gametes; so they cannot be distinguished by examining the types of gametes produced.

Nevertheless, they can often be distinguished by the frequencies of types of gametes: inter-chromosomal recombination produces 50% non-recombinant gametes and 50% recombinant gametes, whereas intra-chromosomal recombination frequently produces less than 50% recombinant gametes. However, when the genes are very far apart on the same chromosome, intra-chromosomal recombination also produces 50% recombinant gametes. The two mechanisms are then genetically indistinguishable.

The Physical Basis of Recombination

William Sutton's chromosome theory of inheritance, which stated that genes are physically located on chromosomes, was supported by Nettie Stevens and Edmund Wilson's discovery that sex was associated with a specific chromosome in insects and Calvin Bridges' demonstration that non-disjunction of X chromosomes was related to the inheritance of eye color in Drosophila. Further evidence for the chromosome theory of heredity came in 1931, when Harriet Creighton and Barbara McClintock (FIGURE 8) obtained evidence that intra-chromosomal recombination was the result of physical exchange between chromosomes. Creighton and McClintock discovered a strain of corn that had an abnormal chromosome 9, containing a densely staining knob at one end and a small piece of another chromosome attached to the other end. This aberrant chromosome allowed them to visually distinguish the two members of a homologous pair.

They studied the inheritance of two traits in corn determined by genes on chromosome 9: at one locus, a dominant allele (C) produced colored kernels, whereas a recessive allele (c) produced colorless kernels; at another, linked locus, a dominant allele (Wx) produced starchy kernels, whereas a recessive allele (wx) produced waxy kernels. Creighton and McClintock obtained a plant that was heterozygous at both loci in repulsion, with the alleles for colored and waxy on the aberrant chromosome and the alleles for colorless and starchy on a normal chromosome:



They crossed this heterozygous plant with a plant that was homozygous for colorless and heterozygous for waxy:

$$\frac{C \quad wx}{c \quad Wx} \times \frac{c \quad Wx}{c \quad wx}$$

This cross will produce different combinations of traits in the progeny, but the only way that colorless and waxy

progeny can arise is through crossing over in the doubly heterozygous parent:

Notice that, if crossing over entails physical exchange between the chromosomes, then the colorless, waxy progeny resulting from recombination should have a chromosome with an extra piece, but not a knob. Furthermore, some of the colored, starchy progeny should possess a knob but not the extra piece. This outcome is precisely what Creighton and McClintock observed, confirming the chromosomal theory of inheritance. Curt Stern provided a similar demonstration by using chromosomal markers in Drosophila at about the same time.

Predicting the Outcomes of Crosses with Linked Genes

Knowing the arrangement of alleles on a chromosome allows us to predict the types of progeny that will result from a cross entailing linked genes and to determine which of these types will be the most numerous. Determining the proportions of the types of offspring requires an additional piece of information—the recombination frequency. The recombination frequency provides us with information about how often the alleles in the gametes appear in new combinations and allows us to predict the proportions of offspring phenotypes that will result from a specific cross entailing linked genes.

In cucumbers, smooth fruit (t) is recessive to warty fruit (T) and glossy fruit (d) is recessive to dull fruit (D). Geneticists have determined that these two genes exhibit a recombination frequency of 16%. Suppose we cross a plant homozygous for warty and dull fruit with a plant homozygous for smooth and glossy fruit and then carry out a testcross by using the F1

$$\frac{T \quad D}{t \quad d} \times \frac{t \quad d}{t \quad d}$$

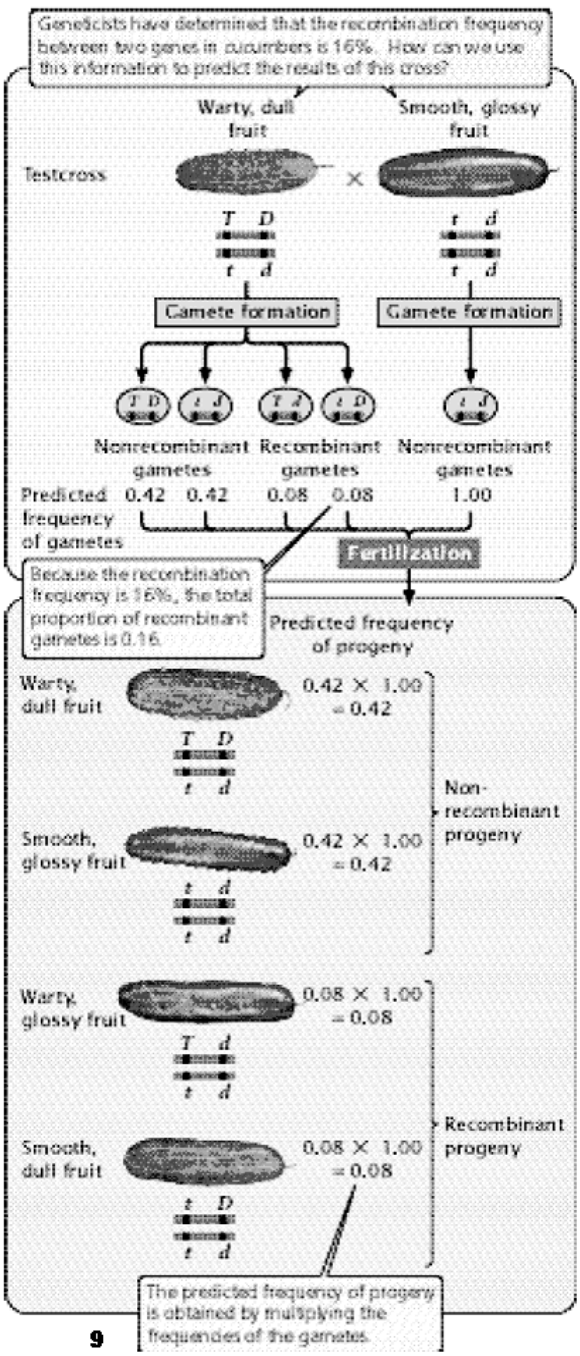
What types and proportions of progeny will result from this testcross?

Four types of gametes will be produced by the heterozygous parent, as shown in (FIGURE 9): two types of nonrecombinant gametes (T D and t d) and two types of recombinant gametes (T d and t D). The recombination frequency tells us that 16% of the gametes produced by the heterozygous parent will be recombinants. Because there are two types of recombinant gametes, each should arise with a frequency of 16%/2 = 8%. All the other gametes will be nonrecombinants; so they should arise with a frequency of 100% - 16% = 84%. Because there are two types of nonrecombinant gametes, each should arise with a frequency of 84%/2 = 42%. The other parent in the testcross is homozygous and therefore produces only a single type of gamete (t d) with a probability of 1.00.

The progeny of the cross result from the union of two gametes, producing four types of progeny (see Figure 9). The expected proportion of each type can be determined by using the multiplication rule, multiplying together the probability of each uniting gamete. Testcross progeny with warty and dull fruit

$$\frac{T \quad D}{t \quad d}$$

appear with a frequency of 0.42 (the probability of inheriting a gamete with chromosome T D from the heterozygous parent) x 1.00 (the probability of inheriting a gamete with chromosome t d from the recessive parent) = 0.42. The proportions of the other types of F2 progeny can be calculated in a similar manner (see Figure 9). This method can be used for predicting the outcome of any cross with linked genes for which the recombination frequency is known.



Testing for Independent Assortment

In some crosses, the genes are obviously linked because there are clearly more non recombinants than recombinants. In other crosses, the difference between independent assortment and linkage is not so obvious. For example, suppose we did a testcross for two pairs of genes, such as AaBb x aabb, and observed the following numbers of progeny: 54 AaBb, 56 aabb, 42 Aabb, and 48 aaBb. Is this outcome a 1:1:1:1 ratio? Not exactly, but it's pretty close. Perhaps these genes are assorting independently and chance produced the slight deviations between the observed numbers and the expected 1:1:1:1 ratio. Alternatively, the genes might be linked, with considerable crossing over taking place between them, and so the number of non recombinants is only slightly greater than the number of recombinants.

How do we distinguish between the roles of chance and of linkage in producing deviations from the results expected with independent assortment?

We encountered a similar problem in crosses in which genes were unlinked—the problem of distinguishing between deviations due to chance and those due to other factors. We addressed this problem with the goodness-of-fit chi-square test, which serves to evaluate the likelihood that chance alone is responsible for deviations between observed and expected numbers. The chi-square test can also be used to test the goodness of fit between observed numbers of progeny and the numbers expected with independent assortment.

Testing for independent assortment between two linked genes requires the calculation of a series of three chi-square tests. To illustrate this analysis, we will examine the data from a cross between German cockroaches, in which yellow body (y) is recessive to brown body (y+) and curved wings (cv) are recessive to straight wings (cv^+). A testcross (y^+ y cv^+ cv x yy cvcv) produced the following progeny:

63	$y^+y\ cv^+cv$	brown body, straight wings
77	$yy\ cvcv$	yellow body, curved wings
28	$y^+y\ cvcv$	brown body, curved wings
32	$yy\ cv^+cv$	yellow body, straight wings
200	total progeny	

Testing ratios at each locus To determine if the genes for body color and wing shape are assorting independently, we must examine each locus separately and determine whether the observed numbers differ from the expected (we will consider why this step is necessary at the end of this section). At the first locus (for body color), the cross between heterozygote and homozygote (y^+ y X yy) is expected to produce y^+ y brown and yy yellow progeny; so we expect 100 of each. We observe 63 +28 = 91 brown progeny and 77 + 32 = 109 yellow progeny. Applying the chi-square test to these observed and expected numbers, we obtain:

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

$$\chi^2 = \frac{(91 - 100)^2}{100} + \frac{(109 - 100)^2}{100}$$

$$= \frac{81}{100} + \frac{81}{100} = 0.81 + 0.81 = 1.62$$

The degrees of freedom associated with the chi-square test are n - 1, where n equals the number of expected classes. Here, there are two expected phenotypes; so the degree of freedom is 2 -1 = 1. Looking up our calculated chi-square value in Table 3.4, we find that the probability associated with this chi-square value is between .30 and .20. Because the probability is above .05 (our critical probability for rejecting the hypothesis that chance produces the difference between observed and expected values), we conclude that there is no significant difference between the 1:1 ratio that we expect in the progeny of the testcross and the ratio that we observed.

We next compare the observed and expected ratios for the second locus, which determines the type of wing. At this locus, a heterozygote and homozygote also were crossed (cv^+ cv X cvcv) and are expected to produce ½ cv^+ cv straight-winged progeny and ½ cvcv curved-wing progeny. We actually observe 63 + 32 = 95 straight-winged progeny and 77 + 28 = 105 curved-wing progeny; so the calculated chi-square value is:

$$\chi^2 = \frac{(95 - 100)^2}{100} + \frac{(105 - 100)^2}{100}$$

$$= \frac{25}{100} + \frac{25}{100} = 0.25 + 0.25 = 0.50$$

The degree of freedom associated with this chi-square value also is 2 - 1 = 1, and the associated probability is between .5 and .3.We again assume that there is no significant difference between what we observed and what we expected at this locus in the testcross. Testing ratios for independent assortment We are now ready to test for the independent assortment of genes at the two loci. If the genes are assorting independently, we can use the multiplication rule to obtain the probabilities and numbers of progeny inheriting different combinations of phenotypes:

Geno-types	Expected pheno-types	Expected propor-tions	Expected numbers	Observed numbers
$y^+y\ cv^+\ cv$	brown, straight	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	50	63
$yy\ cvcv$	yellow, curved	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	50	77
$y^+y\ cvcv$	brown, curved	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	50	28
$yy\ cv^+\ cv$	yellow, straight	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	50	32

The observed and expected numbers of progeny can now be compared by using the chi-square test:

$$\chi^2 = \frac{(63-50)^2}{50} + \frac{(77-50)^2}{50} + \frac{(28-50)^2}{50} + \frac{(32-50)^2}{50} = 34.12$$

Here, we have four expected classes of phenotypes; so the degrees of freedom equal 4 -1 = 3 and the associated probability is considerably less than .001. This very small probability indicates that the phenotypes are not in the proportions that we would expect if independent assortment were taking place. Our conclusion, then, is that these genes are not assorting independently and must be linked.

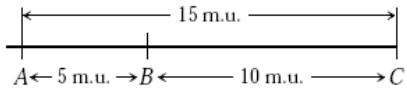
In summary, testing for linkage between two genes requires a series of chi-square tests: a chi-square test for the segregation of alleles at each individual locus, followed by a test for independent assortment between alleles at the different loci. The chi-square tests for segregation at individual loci should always be carried out before testing for independent assortment, because the probabilities expected with independent assortment are based on the probabilities expected at the separate loci. Suppose that the genes in the cockroach example were assorting independently and that some of the cockroaches with curved wings died in embryonic development; the observed proportion with curved wings was then 1/3 instead of ½ . In this case, the proportion of offspring with yellow body and curved wings expected under independent assortment should be 1/3 x1/2 =1/6 instead of ¼ . Without the initial chi-square test for segregation at the curved-wing locus, we would have no way of knowing that what we expected with independent assortment was 1/6 instead of ¼ . If we carried out only the final test for independent assortment and assumed an expected 1:1:1:1 ratio, we would obtain a high chi square value. We might conclude, erroneously, that the genes were linked.

If a significant chi-square (one that has a probability less than 0.05) is obtained in either of the first two tests for segregation, then the final chi-square for independent assortment should not be carried out, because the true expected values are unknown.

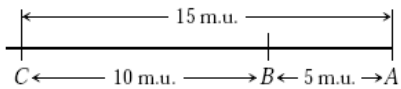
Gene Mapping with Recombination Frequencies

Morgan and his students developed the idea that physical distances between genes on a chromosome are related to the rates of recombination. They hypothesized that crossover events occur more or less at random up and down the chromosome and that two genes that lie far apart are more likely to undergo a crossover than are two genes that lie close together. They proposed that recombination frequencies could provide a convenient way to determine the order of genes along a chromosome and would give estimates of the relative distances between the genes. Chromosome maps calculated by using recombination frequencies are called genetic maps. In contrast, chromosome maps based on physical distances along the chromosome (often expressed in terms of numbers of base pairs) are called physical maps.

Distances on genetic maps are measured in map units (abbreviated m.u.); one map unit equals 1% recombination. Map units are also called centimorgans (cM), in honor of Thomas Hunt Morgan; one morgan equals 100 m.u. Genetic distances measured with recombination rates are approximately additive: if the distance from gene A to gene B is 5 m.u., the distance from gene B to gene C 10 m.u., and the distance from gene A to gene C is 15 m.u., gene B must be located between genes A and C. On the basis of the map distances just given, we could draw a simple genetic map for genes A, B, and C, as shown here:



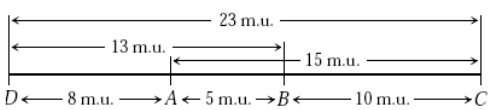
We could just as plausibly draw this map with C on the left and A on the right:



Both maps are correct and equivalent because, with information about the relative positions of only three genes, the most that we can determine is which gene lies in the middle. If we obtained distances to an additional gene, then we could position A and C relative to that gene. An additional gene D, examined through genetic crosses, might yield the following recombination frequencies:

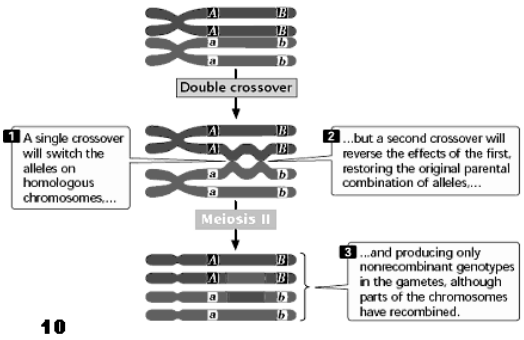
Gene pair	Recombination frequency (%)
A and *D*	8
B and *D*	13
C and *D*	23

Notice that C and D exhibit the greatest amount of recombination; therefore, C and D must be farthest apart, with genes A and B between them. Using the recombination frequencies and remembering that 1 m.u. = 1% recombination, we can now add D to our map:



By doing a series of crosses between pairs of genes, we can construct genetic maps showing the linkage arrangements of a number of genes. Two points should be emphasized about constructing chromosome maps from recombination frequencies. First, recall that the recombination frequency between two genes cannot exceed 50% and that 50% is also the rate of recombination for genes located on different chromosomes. Consequently, one cannot distinguish between genes on different chromosomes and genes located far apart on the same chromosome. If genes exhibit 50% recombination, the most that can be said about them is that they belong to different groups of linked genes (different

linkage groups), either on different chromosomes or far apart on the same chromosome.



A double crossover between two linked genes produces only nonrecombinant gametes.

A second point is that a testcross for two genes that are relatively far apart on the same chromosome tends to underestimate the true recombination frequency, because the cross does not reveal double crossovers that might take place between the two genes (FIGURE 10). A double crossover arises when two separate crossover events take place between the same two loci. Whereas a single crossover switches the alleles on the homologous chromosomes—producing combinations of alleles that were not present on the original parental chromosomes—a second crossover between the same two genes reverses the effects of the first, thus restoring the original parental combination of alleles. Double crossovers produce only non recombinant gametes, and we cannot distinguish between the progeny produced by double crossovers and the progeny produced when there is no crossing over. However, as we shall see in the next section, it is possible to detect double crossovers if we examine a third gene that lies between the two crossovers. Because double crossovers between two genes go undetected, recombination frequencies will be underestimated whenever double crossovers take place.

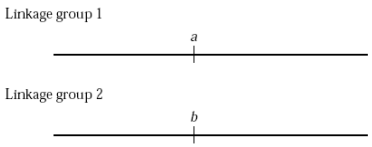
Double crossovers are more frequent between genes that are far apart; therefore genetic maps based on short distances are always more accurate than those based on longer distances.

Constructing a Genetic Map with Two-Point Testcrosses

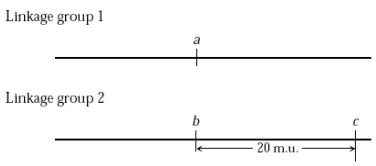
Genetic maps can be constructed by conducting a series of testcrosses between pairs of genes and examining the recombination frequencies between them. A testcross between two genes is called a two-point testcross or a two-point cross for short. Suppose that we carried out a series of two-point crosses for four genes, a, b, c, and d, and obtained the following recombination frequencies:

Gene loci in testcross	Recombinant frequency (%)
a and b	50
a and c	50
a and d	50
b and c	20
b and d	10
c and d	28

We can begin constructing a genetic map for these genes by considering the recombination frequencies for each pair of genes. The recombination frequency between a and b is 50%, which is the recombination frequency expected with independent assortment. Genes a and b may therefore either be on different chromosomes or be very far apart on the same chromosome; so we will place them in different linkage groups with the understanding that they may or may not be on the same chromosome:

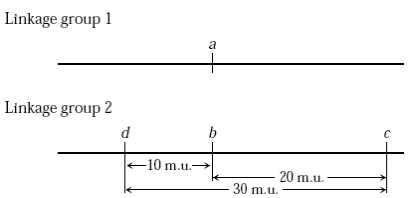


The recombination frequency between a and c is 50%, indicating that they, too, are in different linkage groups. The recombination frequency between b and c is 20%; so these genes are linked and separated by 20 map units:



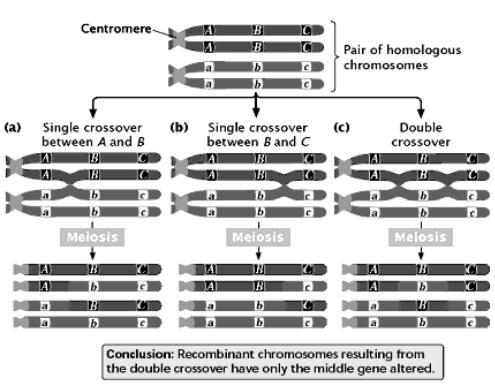
The recombination frequency between a and d is 50%, indicating that these genes belong to different linkage groups, whereas genes b and d are linked, with a recombination frequency of 10%. To decide whether gene d is 10 map units to the left or right of gene b, we must consult the c-to-d distance. If gene d is 10 map units to the left of gene b, then the distance between d and c should be 20 m.u. + 10 m.u. = 30 m.u. This distance will be only approximate because any double crossovers between the two genes will be missed and the recombination frequency will be underestimated.

If, on the other hand, gene d lies to the right of gene b, then the distance between gene d and c will be much shorter, approximately 20 m.u. - 10 m.u. = 10 m.u. By examining the recombination frequency between c and d, we can distinguish between these two possibilities. The recombination frequency between c and d is 28%; so gene d must lie to the left of gene b. Notice that the sum of the recombination between d and b (10%) and between b and c (20%) is greater than the recombination between d and c (28%). (This is what was meant by saying that recombination rates—i.e., map units—are approximately additive.) This discrepancy arises because double crossovers between the two outer genes go undetected, causing an underestimation of the true recombination frequency. The genetic map of these genes is now complete:



Linkage and Recombination Between Three Genes

Genetic maps can be constructed from a series of testcrosses for pairs of genes, but this approach is not particularly efficient, because numerous two-point crosses must be carried out to establish the order of the genes and because double crossovers are missed. A more efficient mapping technique is a testcross for three genes (a three-point testcross, or three point cross).With a three-point cross, the order of the three genes can be established in a single set of progeny and some double crossovers can usually be detected, providing more accurate map distances.



11 Three types of crossovers can take place among three linked loci.

Consider what happens when crossing over takes place among three hypothetical linked genes. FIGURE 11 illustrates a pair of homologous chromosomes from an individual that is heterozygous at three loci (AaBbCc). Notice that the genes are in the coupling configuration; that is, all the dominant alleles are on one chromosome (A B C) and all the recessive alleles are on the other chromosome (a b c). Three types of crossover events can take place between these three genes: two types of single crossovers (see Figure 11a and b) and a double crossover (see Figure 11c). In each type of crossover, two of the resulting chromosomes are recombinants and two are non recombinants. Notice that, in the recombinant chromosomes resulting from the double crossover, the outer two alleles are the same as in the non recombinants, but the middle allele is different. This result provides us with an important clue about the order of the genes. In progeny that result from a double crossover, only the middle allele should differ from the alleles present in the non-recombinant progeny.

Gene Mapping with the Three-Point Testcross

To examine gene mapping with a three-point testcross, we will consider three recessive mutations in the fruit fly Drosophila melanogaster. In this species, scarlet eyes (st) are recessive to red eyes (st^+), ebony body color (e) is recessive to gray body color (e^+), and spineless (ss)—that is, the presence of small bristles—is recessive to normal bristles (ss^+). All three mutations are linked and located on the third chromosome.

We will refer to these three loci as st, e, and ss, but keep in mind that either recessive alleles (st, e, and ss) or the dominant alleles (st^+, e^+, and ss^+) may be present at each locus. So, when we say that there are 10 m.u. between st and ss, we mean that there are 10 m.u. between the loci at which these mutations occur; we could just as easily say that there are 10 m.u. between st^+ and ss^+. To map these genes, we need to determine their order on the chromosome and the genetic distances between them. First, we must set up a three-point testcross, a cross between a fly heterozygous at all three loci and a fly homozygous for recessive alleles at all three loci. To produce flies heterozygous for all three loci, we might cross a stock of flies that are homozygous for normal alleles at all three loci with flies that are homozygous for recessive alleles at all three loci:

$$\text{P} \quad \frac{st^+ \quad e^+ \quad ss^+}{st^+ \quad e^+ \quad ss^+} \times \frac{st \quad e \quad ss}{st \quad e \quad ss}$$

$$\text{F}_1 \quad \frac{st^+ \quad e^+ \quad ss^+}{st \quad e \quad ss}$$

The order of the genes has been arbitrarily assigned because at this point we do not know which is the middle gene. Additionally, the alleles in these heterozygotes are in coupling configuration (because all the wild-type dominant alleles were inherited from one parent and all the recessive mutations from the other parent), although the testcross can also be done with genes in repulsion. In the three-point testcross, we cross the F1 heterozygotes with flies that are homozygous for all three recessive mutations. In many organisms, it makes no difference whether the heterozygous parent in the testcross is male or female (provided that the genes are autosomal) but, in Drosophila, no crossing over takes place in males. Because crossing over in the heterozygous parent is essential for determining recombination frequencies, the heterozygous flies in our testcross must be female. So we mate female F1 flies that are heterozygous for all three traits with male flies that are homozygous for all the recessive traits:

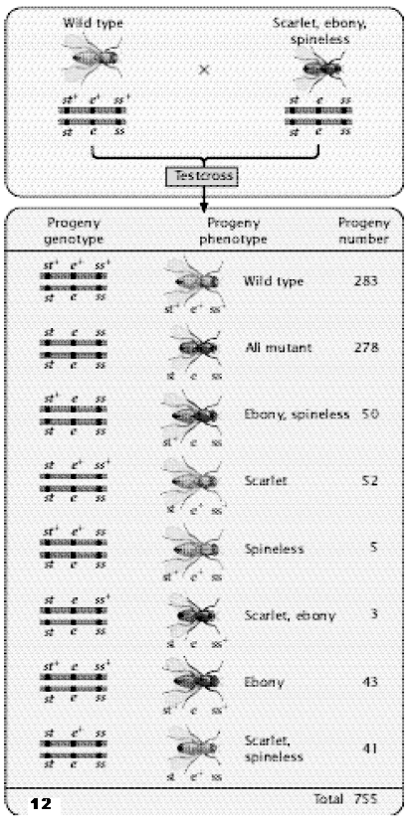
$$\frac{st^+ \quad e^+ \quad ss^+}{st \quad e \quad ss}\ \text{female} \times \frac{st \quad e \quad ss}{st \quad e \quad ss}\ \text{male}$$

The progeny produced from this cross are listed in FIGURE 12. For each locus, two classes of progeny are produced: progeny that are heterozygous, displaying the dominant trait, and progeny that are homozygous, displaying the recessive trait. With two classes of progeny possible for each of the three loci, there will be $2^3 = 8$ classes of phenotypes possible in the progeny. In this example, all eight phenotypic classes are present but, in some three-point crosses, one or more of the phenotypes may be missing if the number of progeny is limited. Nevertheless, the absence of a particular class can provide important information about which combination of traits is least frequent and ultimately the order of the genes, as we will see.

To map the genes, we need information about where and how often crossing over has occurred. In the homozygous recessive parent, the two alleles at each locus are the same; and so crossing over will have no effect on the types of gametes produced; with or without crossing over, all gametes from this parent have a chromosome with three recessive alleles (st e ss). In contrast, the heterozygous

parent has different alleles on its two chromosomes; so crossing over can be detected. The information that we need for mapping, therefore, comes entirely from the gametes produced by the heterozygous parent. Because chromosomes contributed by the homozygous parent carry only recessive alleles, whatever alleles are present on the chromosome contributed by the heterozygous parent will be expressed in the progeny.

As a shortcut, we usually do not write out the complete genotypes of the testcross progeny, listing instead only the alleles expressed in the phenotype (as shown in Figure 12), which are the alleles inherited from the heterozygous parent.



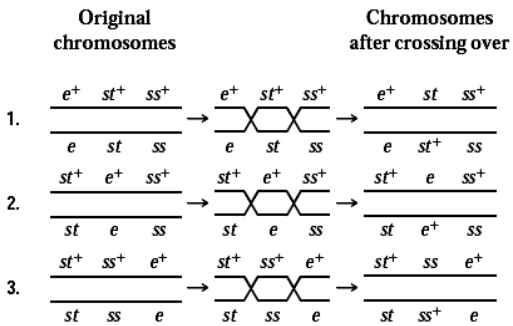
Determining the gene order The first task in mapping the genes is to determine their order on the chromosome. In Figure 12, we arbitrarily listed the loci in the order st, e, ss, but we had no way of knowing which of the three loci was between the other two. We can now identify the middle locus by examining the double-crossover progeny.

First, determine which progeny are the non recombinants—they will be the two most-numerous classes of progeny. (Even if crossing over takes place in every meiosis, the non recombinants will comprise at least 50% of the progeny.)

Among the progeny of the testcross in Figure 13, the most numerous are those with all three dominant traits (st^+ e^+ ss^+) and those with all three recessive traits (st e ss).

Next, identify the double-crossover progeny. These should always be the two least-numerous phenotypes, because the probability of a double crossover is always less than the probability of a single crossover. The least-common progeny among those listed in Figure 12 are progeny with red eyes, gray body, and spineless bristles (st^+ e^+ ss) and progeny with scarlet eyes, ebony body, and normal bristles (st e ss^+); so they are the double-crossover progeny.

Three orders of genes are possible: the eye-color locus could be in the middle (e st ss), the body-color locus could be in the middle (st e ss), or the bristle locus could be in the middle (st ss e). To determine which gene is in the middle, we can draw the chromosomes of the heterozygous parent with all three possible gene orders and then see if a double crossover produces the combination of genes observed in the double crossover progeny. The three possible gene orders and the types of progeny produced by their double crossovers are:

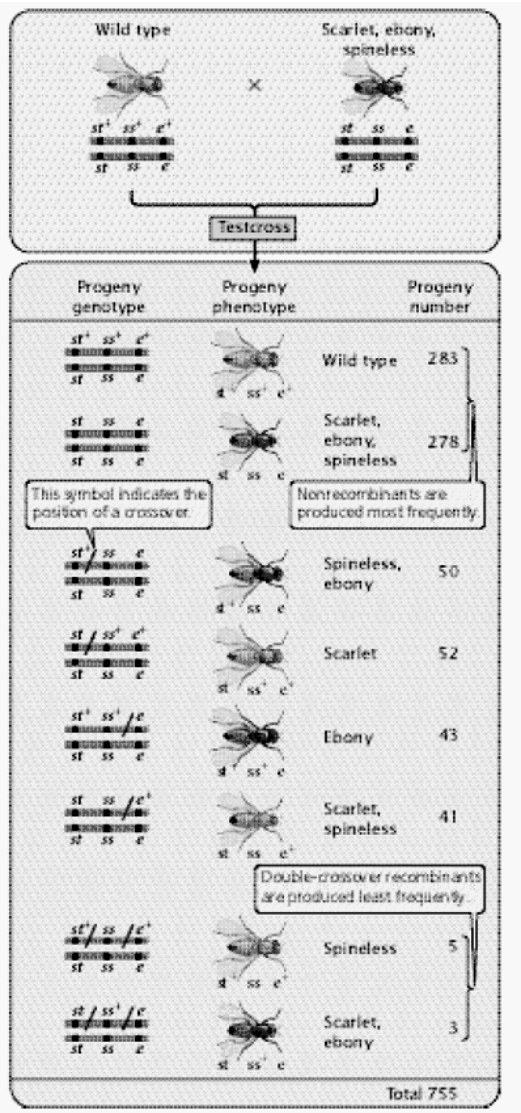


The only gene order that produces chromosomes with alleles for the traits observed in the double crossovers (st^+ e^+ ss and st e ss^+) is the third one, where the locus for bristle shape lies in the middle. Therefore, this order (st ss e) must be the correct sequence of genes on the chromosome.

With a little practice, it's possible to quickly determine which locus is in the middle without writing out all the gene orders. The phenotypes of the progeny are expressions of the alleles inherited from the heterozygous parent. Recall that, when we looked at the results of double crossovers (see Figure 12), only the alleles at the middle locus differed from the non recombinants. If we compare the non recombinant progeny with double-crossover progeny, they should differ only in alleles of the middle locus.

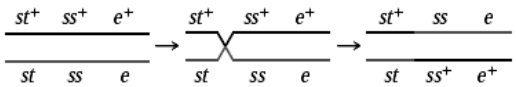
Let's compare the alleles in the double-crossover progeny st^+ e^+ ss with those in the nonrecombinant progeny st^+ e^+ ss^+. We see that both have an allele for red eyes (st^+) and both have an allele for gray body (e^+), but the non recombinants have an allele for normal bristles (ss^+), whereas the double crossovers have an allele for spineless bristles (ss). Because the bristle locus is the only one that differs, it must lie in the middle. We would obtain the same results if we compared the other class of double-crossover progeny (st e ss^+) with other nonrecombinant progeny (st e ss). Again the only trait that differs is the one for bristles. Don't forget that the nonrecombinants and the double crossovers should differ only at one locus; if they differ in two loci, the wrong classes of progeny are being compared.

Determining the locations of crossovers When we know the correct order of the loci on the chromosome, we should rewrite the phenotypes of the testcross progeny in Figure 13 with the loci in the correct order so that we can determine where crossovers have taken place (FIGURE 13). Among the eight classes of progeny, we have already identified two classes as non-recombinants (st^+ ss^+ e^+ and st ss e) and two classes as double crossovers (st^+ ss e^+ and st ss^+ e). The other four classes include progeny that resulted from a chromosome that underwent a single crossover: two underwent single crossovers between st and ss, and two underwent single crossovers between ss and e.



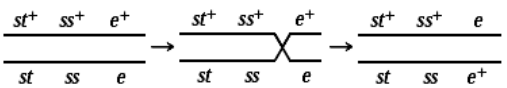
13 Writing the results of a three-point testcross with the loci in the correct order allows the locations of crossovers to be determined.

To determine where the crossovers took place in these progeny, compare the alleles found in the single-crossover progeny with those found in the non recombinants, just as we did for the double crossovers. Some of the alleles in the single crossover progeny are derived from one of the original (non recombinant) chromosomes of the heterozygous parent, but at some place there is a switch (due to crossing over) and the remaining alleles are derived from the homologous non recombinant chromosome. The position of the switch indicates where the crossover event took place. For example, consider progeny with chromosome st^+ ss e . The first allele (st^+) came from the nonrecombinant chromosome st^+ ss^+ e^+ and the other two alleles (ss and e) must have come from the other nonrecombinant chromosome st ss e through crossing over:



This same crossover also produces the st ss^+ e^+ progeny.

This same method can be used to determine the location of crossing over in the other two types of single crossover progeny. Crossing between ss and e produces st^+ ss^+ e and st ss e^+ chromosomes:



We now know the locations of all the crossovers; their locations are marked with a slash in Figure 13.

Calculating the recombination frequencies Next, we can determine the map distances, which are based on the frequencies of recombination. Recombination frequency is calculated by adding up all of the recombinant progeny, dividing this number by the total number of progeny from the cross, and multiplying the number obtained by 100%. To determine the map distances accurately, we must include all crossovers (both single and double) that take place between two genes.

Recombinant progeny that possess a chromosome that underwent crossing over between the eye-color locus (st) and the bristle locus (ss) include the single crossovers (st^+ / ss e and st / ss^+ e^+) and the two double crossovers (st^+ / ss / e^+ and st / ss^+ / e); see Figure 13. There are a total of 755 progeny; so the recombination frequency between ss and st is:

$$st\text{–}ss \text{ recombination frequency} = \frac{(50 + 52 + 5 + 3)}{755} \times 100\% = 14.6\%$$

The distance between the st and ss loci can be expressed as 14.6 m.u.

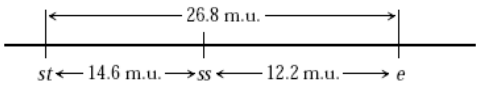
The map distance between the bristle locus (ss) and the body locus (e) is determined in the same manner. The recombinant progeny that possess a crossover between ss and e are the single crossovers st^+ ss^+ / e and st ss / e^+, and the double crossovers st^+ / ss / e^+ and st / ss^+ / e . The recombination frequency is:

$$ss\text{–}e \text{ recombination frequency} = \frac{(43 + 41 + 5 + 3)}{755} \times 100\% = 12.2\%$$

Thus, the genetic distance between ss and e is 12.2 m.u. Finally, calculate the genetic distance between the outer two loci, st and e. Add up all the progeny with crossovers between the two loci. These progeny include those with a single crossover between st and ss, those with a single crossover between ss and e, and the double crossovers ($\underline{st^+ / ss / e^+}$ and $\underline{st / ss^+ / e}$). Because the double crossovers have two crossovers between st and e, we must add the double crossovers twice to determine the recombination frequency between these two loci:

$$st\text{–}e \text{ recombination frequency} = \frac{(50 + 52 + 43 + 41 + (2 \times 5) + (2 \times 3)}{755} \times 100\% = 26.8\%$$

Notice that the distances between st and ss (14.6 m.u.) and between ss and e (12.2 m.u.) add up to the distance between st and e (26.8 m.u.). We can now use the map distances to draw a map of the three genes on the chromosome:



Interference and coefficient of coincidence Map distances give us information not only about the physical distances that separate genes, but also about the proportions of recombinant and non recombinant gametes that will be produced in a cross. For example, knowing that genes st and ss on the third chromosome of D. melanogaster are separated by a distance of 14.6 m.u. tells us that 14.6% of the gametes produced by a fly heterozygous at these two loci will be recombinants. Similarly, 12.2% of the gametes from a fly heterozygous for ss and e will be recombinants.

Theoretically, we should be able to calculate the proportion of double-recombinant gametes by using the multiplication rule of probability, which states that the probability of two independent events occurring together is the multiplication of their independent probabilities.

Applying this principle, we should find that the proportion (probability) of gametes with double crossovers between st and e is equal to the probability of recombination between st and ss, multiplied by the probability of recombination between ss and e, or 0.146 x 0.122 = 0.0178. Multiplying this probability by the total number of progeny gives us the expected number of double-crossover progeny from the cross: 0.0178 x 755 = 13.4. Only 8 double crossovers—considerably fewer than the 13 expected— were observed in the progeny of the cross.

This phenomenon is common in eukaryotic organisms. The calculation assumes that each crossover event is independent and that the occurrence of one crossover does not influence the occurrence of another. But crossovers are frequently not independent events: the occurrence of one tends to inhibit additional crossovers in the same region of the chromosome, and so double crossovers are less frequent than expected.

The degree to which one crossover interferes with additional crossovers in the same region is termed the interference.

To calculate the interference, we first determine the together is the multiplication of their independent probabilities. Applying this principle, we should find that the proportion (probability) of gametes with double crossovers between st and e is equal to the probability of recombination between st and ss, multiplied by the probability of recombination between ss and e, or 0.146 - 0.122 = 0.0178. Multiplying this probability by the total number of progeny gives us the expected number of double-crossover progeny from the cross: 0.0178 x 755 = 13.4. Only 8 double crossovers—considerably fewer than the 13 expected—were observed in the progeny of the cross (see Figure 12).

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The degree to which one crossover interferes with additional crossovers in the same region is termed the interference. To calculate the interference, we first determine the

$$\text{coefficient of coincidence} = \frac{\text{number of observed double crossovers}}{\text{number of expected double crossovers}}$$

For the loci that we mapped on the third chromosome of D. melanogaster (see Figure 13), we find that:

$$\text{coefficient of coincidence} = \frac{5 + 3}{0.146 \times 0.122 \times 755} = \frac{8}{13.4} = 0.6$$

which indicates that we are actually observing only 60% of the double crossovers that we expected on the basis of the single-crossover frequencies. The interference is calculated as: interference - 1 = coefficient of coincidence So the interference for our three-point cross is:

interference = 1 - 0.6 = 0.4

This value of interference tells us that 40% of the double crossover progeny expected will not be observed because of interference. When interference is complete and no double crossover progeny are observed, the coefficient of coincidence is 0 and the interference is 1. Sometimes more double-crossover progeny appear than expected, which happens when a crossover increases the probability of another crossover occurring nearby. In this case, the coefficient of coincidence is greater than 1 and the interference will be negative.

Gene Mapping in Humans

Efforts in mapping the human genome are hampered by the inability to perform desired crosses and the small number of progeny in most human families. Geneticists are restricted to analyses of pedigrees, which are often incomplete and provide limited information. Nevertheless, techniques have been developed that use pedigree data to analyze linkage, and a large number of human traits have been successfully mapped with the use of these methods.

Because the number of progeny from any one mating is usually small, data from several families and pedigrees are usually combined to test for independent assortment. The methods used in these types of analysis are beyond the scope of this book, but an example will illustrate how linkage can be detected from pedigree data.

One of the first documented demonstrations of linkage in humans was between the locus for nail–patella syndrome and the locus that determines the ABO blood types. Nail–patella syndrome is an autosomal dominant disorder characterized by abnormal fingernails and absent or rudimentary kneecaps. The ABO blood types are determined by an autosomal locus with multiple alleles. Linkage between the genes encoding these traits was established in families in which both traits segregate. Part of one such family is illustrated in FIGURE 14.

Nail–patella syndrome is relatively rare; so we can assume that people having this trait are heterozygous (Nn); unaffected people are homozygous (nn). The ABO genotypes can be inferred from the phenotypes and the types of offspring produced. Person I-2 in Figure 14, for example, has blood type B, which has two possible genotypes: I^BI^B or I^Bi. Because some of her offspring are blood type O (genotype ii) and must therefore have inherited an i allele from each parent, female I-2 must have genotype I^B i. Similarly, the presence of blood type O offspring in generation II indicates that male I-1, with blood type A, also must carry an i allele and therefore has genotype I^Ai. The ABO and nail–patella genotypes for all persons in the pedigree are given below the squares and circles. From generation II, we can see that the genes for nail–patella syndrome and the blood types do not appear to assort independently. The parents of this family are:

I^A i Nn x I^Bi nn

If the genes coding for nail–patella syndrome and the ABO blood types assorted independently, we would expect that some children in generation II would have blood type A and nail–patella syndrome, inheriting both the I^A and N genes from their father. However, all children in generation II with nail–patella syndrome have either blood type B or blood type O; all those with blood type A have normal nails and kneecaps. This outcome indicates that the arrangements of the alleles on the chromosomes of the crossed parents are:

$$\frac{I^A \quad n}{i \quad N} \times \frac{I^B \quad n}{i \quad n}$$

There is no recombination among the offspring of these parents (generation II), but there are two instances of recombination among the persons in generation III. Individuals II-1 and II-2 have the following genotypes:

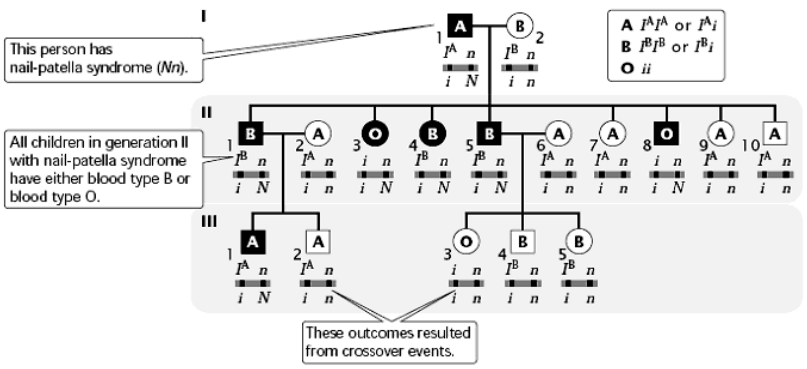
$$\frac{I^B \quad n}{i \quad N} \times \frac{I^A \quad n}{i \quad n}$$

Their child III-2 has blood type A and does not have nail–patella syndrome; so he must have genotype:

$$\frac{I^A \quad n}{i \quad n}$$

and must have inherited both the i and the n alleles from his father. These alleles are on different chromosomes in the father; so crossing over must have taken place. Crossing over also must have taken place to produce child III-3.

In the pedigree of Figure 14, there are 12 children from matings in which the genes encoding nail–patella syndrome and ABO blood types segregate; 2 of them are recombinants. On this basis, we might assume that the loci for nail–patella syndrome and ABO blood types are linked, with a recombination frequency of 2/12 = 0.167. However, it is possible that the genes are assorting independently and that the small number of children just makes it seem as though the genes are linked. To determine the probability that genes are actually linked, geneticists often calculate lod (logarithm of odds) scores.



14 Linkage between ABO blood types and nail–patella syndrome was established by examining families in whom both traits segregate. The pedigree shown here is for one such family. Solid circles and squares represent the presence of nail–patella syndrome; the ABO blood type is indicated in each circle or square. The genotype, inferred from phenotype, is given below each square or circle.

To obtain a lod score, one calculates both the probability of obtaining the observations with a specified degree of linkage and the probability of obtaining the observations with independent assortment. One then determines the ratio of these two probabilities, and the logarithm of this ratio is the lod score. Suppose that the probability of obtaining a particular set of observations with linkage and a certain recombination frequency is 0.1 and that the probability of obtaining the same observations with independent assortment is 0.0001. The ratio of these two probabilities is

0.1/0.0001 = 1000, the logarithm of which (the lod score) is 3. Thus linkage with the specified recombination is 1000 times as likely to produce what was observed as independent assortment. A lod score of 3 or higher is usually considered convincing evidence for linkage.

Mapping with Molecular Markers

For many years, gene mapping was limited in most organisms by the availability of genetic markers, variable genes with easily observable phenotypes whose inheritance could be studied. Traditional genetic markers include genes that encode easily observable characteristics such as flower color, seed shape, blood types, and biochemical differences. The paucity of these types of characteristics in many organisms limited mapping efforts.

In the 1980s, new molecular techniques made it possible to examine variations in DNA itself, providing an almost unlimited number of genetic markers that can be used for creating genetic maps and studying linkage relations.

The earliest of these molecular markers consisted of restriction fragment length polymorphisms (RFLPs), variations in DNA sequence detected by cutting the DNA with restriction enzymes. Later, methods were developed for detecting variable numbers of short DNA sequences repeated in tandem, called variable number of tandem repeats (VNTRs). More recently, DNA sequencing allows the direct detection of individual variations in the DNA nucleotides, called single nucleotide polymorphisms (SNPs). All of these methods have expanded the availability of genetic markers and greatly facilitated the creation of genetic maps.

Gene mapping with molecular markers is done essentially in the same manner as mapping performed with traditional phenotypic markers: the cosegregation of two or more markers is studied and map distances are based on the rates of recombination between markers.

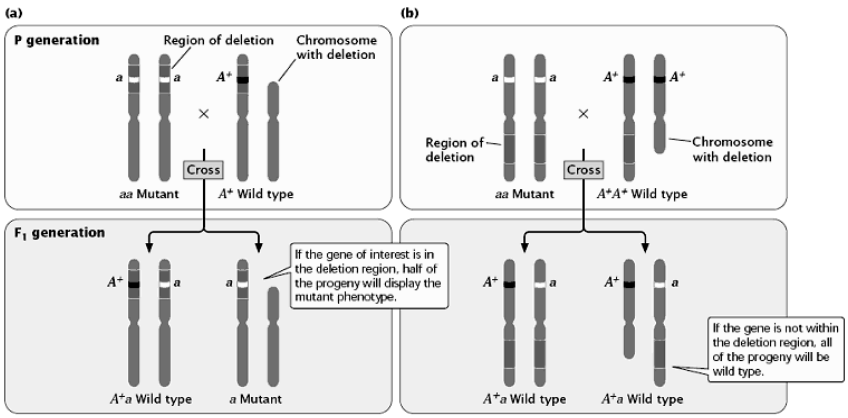
Physical Chromosome Mapping

Genetic maps reveal the relative positions of genes on a chromosome on the basis of frequencies of crossing over, but they do not provide information that can allow us to place groups of linked genes on particular chromosomes. Furthermore, the units of a genetic map do not always precisely correspond to physical distances on the chromosome, because a number of factors other than physical distances between genes (such as the type and sex of the organism) can influence rates of crossing over. Because of these limitations, physical-mapping methods that do not rely on rates of crossing over have been developed.

Deletion Mapping

One method for determining the chromosomal location of a gene is deletion mapping. Special staining methods have been developed that make it possible to detect chromosome deletions, mutations in which a part of a chromosome is missing. Genes are assigned to regions of particular chromosomes by studying the association of a gene's phenotype or product and particular chromosome deletions. In deletion mapping, an individual that is homozygous for a recessive mutation in the gene of interest is crossed with an individual that is heterozygous for a deletion (FIGURE 15). If the gene of interest is in the region of the chromosome represented by the deletion (the red part of chromosome in Figure 15), approximately half of the progeny will display the mutant phenotype (see Figure 15a). If the gene is not within the deleted region, all of the progeny will be wild type (see Figure 15b). Deletion mapping has been used to reveal the chromosomal locations of a number of human genes. For example,

Duchenne muscular dystrophy is a disease that causes progressive weakening and degeneration of the muscles. From its X-linked pattern of inheritance, the mutated allele causing this disorder was known to be on the X chromosome, but its precise location was uncertain. Examination of a number of patients having Duchenne muscular dystrophy, who also possessed small deletions, allowed researchers to position the gene to a small segment of the short arm of the X chromosome.

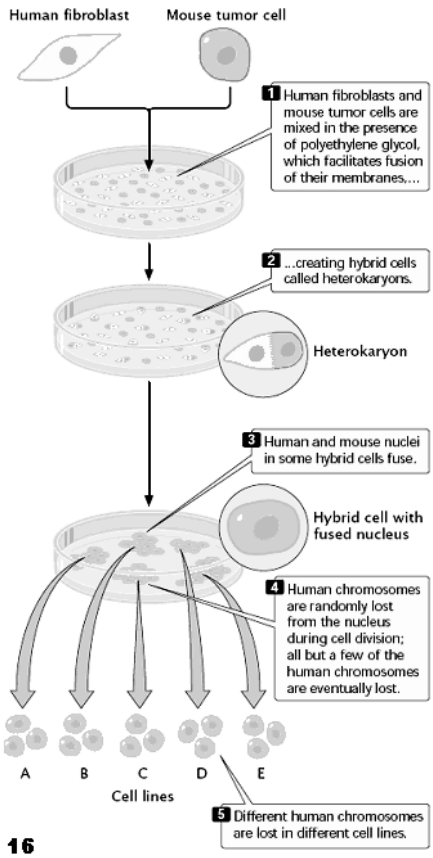


15 Deletion mapping can be used to determine the chromosomal location of a gene.
An individual homozygous for a recessive mutation in the gene of interest (*aa*) is crossed with an individual heterozygous for a deletion.

Somatic-Cell Hybridization

Another method used for positioning genes on chromosomes is somatic cell hybridization, which requires the fusion of different types of cells. Most mature somatic (non-sex) cells can undergo only a limited number of divisions and therefore cannot be grown continuously. However, cells that have been altered by viruses or derived from tumors that have lost the normal constraints on cell division will divide indefinitely; these types of cells can be cultured in the laboratory and are referred to as a cell line.

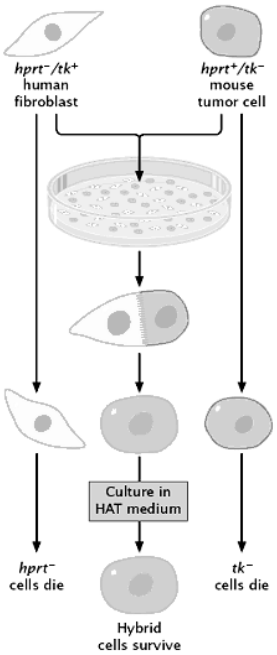
Cells from two different cell lines can be fused by treating them with polyethylene glycol or other agents that alter their plasma membranes. After fusion, the cell possesses two nuclei and is called a heterokaryon. The two nuclei of a heterokaryon eventually also fuse, generating a hybrid cell that contains chromosomes from both cell lines. If human and mouse cells are mixed in the presence of polyethylene glycol, fusion results in human–mouse somatic-cell hybrids (FIGURE 16).



16

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

The hybrid cells tend to lose chromosomes as they divide and, for reasons that are not understood, chromosomes from one of the species are lost preferentially. In human–mouse somatic-cell hybrids, the human chromosomes tend to be lost, whereas the mouse chromosomes are retained. Eventually, the chromosome number stabilizes when all but a few of the human chromosomes have been lost. Chromosome loss is random and differs among cell lines. The presence of these "extra" human chromosomes in the mouse genome makes it possible to assign human genes to specific chromosomes. In the first step of this procedure, hybrid cells must be separated from original parental cells that have not undergone hybridization. This separation is accomplished by using a selection method that allows hybrid cells to grow while suppressing the growth of parental cells. The most commonly used method is called HAT selection (FIGURE 17), which stands for hypoxanthine, aminopterin, and thymidine, three chemicals that are used to select for hybrid cells. In the presence of HAT medium, a cell must possess two enzymes to synthesize DNA: thymidine kinase (TK) and hypoxanthine- guanine phosphoribosyl transferase (HPRT). Cells that are tk^- or hpr^- cannot synthesize DNA and will not grow on HAT medium. The mouse cells used in the hybridization procedure are deficient in TK, but can produce HPRT (the cells are $tk^- hprt^+$); the human cells can produce TK but are deficient for HPRT (they are $tk^+ hprt^-$). On HAT medium, the mouse cells do not survive, because they are tk^-; the human cells do not survive, because they are $hprt^-$. Hybrid cells, on the other hand, inherit the ability to make HPRT from the mouse cell and the ability to make TK from the human cell; thus, they produce both enzymes (the cells are $tk^+ hprt^+$) and will grow on HAT medium.



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

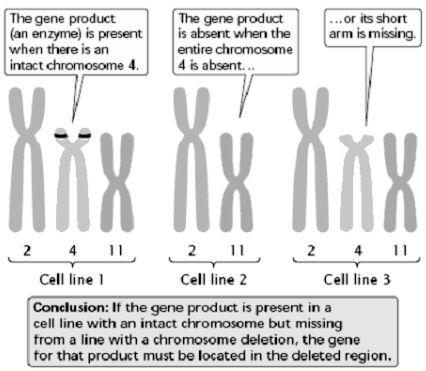
To map genes using somatic-cell hybridization requires the use of a panel of different hybrid cell lines. The cell lines of the panel differ in the human chromosomes that they have retained. For example, one cell line might possess human chromosomes 2, 4, 7, and 8, whereas another might possess chromosomes 4, 19, and 20. Each cell line in the panel is examined for evidence of a particular human gene. The human gene can be detected either by looking for the protein that it produces or by looking for the gene itself with the use of molecular probes. Correlation of the presence of the gene with the presence of specific human chromosomes often allows the gene to be assigned to the correct chromosome. For example, if a gene was detected in both of the aforementioned cell lines, the gene must be on chromosome 4, because it is the only human chromosome common to both cell lines (FIGURE 18).

Two genes determined to be on the same chromosome with the use of somatic-cell hybridization are said to be syntenic genes. This term is used because syntenic genes may or may not exhibit linkage in the traditional genetic sense—remember that two genes can be located on the same chromosome but may be so far apart that they assort independently. Syntenic refers to genes that are physically linked, regardless of whether they exhibit genetic linkage. (Synteny is sometimes also used to refer to gene loci in different organisms located on a chromosome region of common evolutionary origin.) Sometimes somatic-cell hybridization can be used to position a gene on a specific part of a chromosome. Some hybrid cell lines carry a human chromosome with a chromosome mutation such as a deletion or a translocation.

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

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

If the gene is present in a cell line with the intact chromosome but missing from a line with a chromosome deletion, the gene must be located in the deleted region (FIGURE 19) Similarly, if a gene is usually absent from a chromosome but consistently appears whenever a translocation (a piece of another chromosome that has broken off and attached itself to the chromosome in question) is present, it must be present on the translocated part of the chromosome.

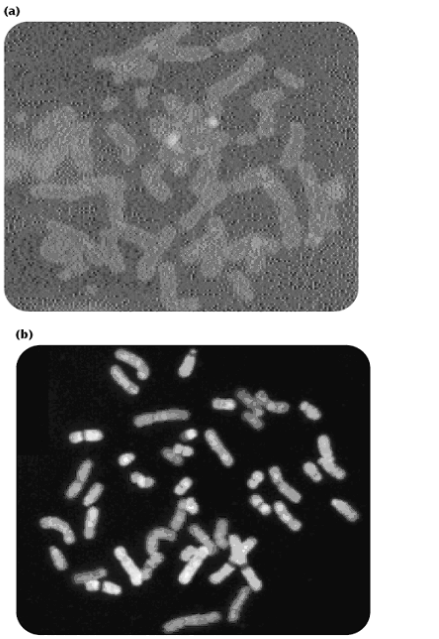


19 Genes can be localized to a specific part of a chromosome by using somatic-cell hybridization.

In Situ Hybridization

In situ hybridization is another method for determining the chromosomal location of a particular gene. This method requires a DNA copy of the gene or its RNA product, which is used to make a molecule (called a probe) that is complementary to the gene of interest. The probe is made radioactive or is attached to a special molecule that fluoresces under ultraviolet (UV) light and is added to chromosomes from specially treated cells that have been spread on a microscope slide. The probe binds to the complementary DNA sequence of the gene on the chromosome. The presence of radioactivity or fluorescence from the bound probe reveals the location of the gene on a particular chromosome (FIGURE 20a).

The use of fluorescence in situ hybridization (FISH) has been widely used to identify the chromosomal location of human genes. In spectral karyotyping (SKY) (FIGURE 20b), a set of 24 FISH probes, each specific to a different human chromosome and attached to a molecule that fluoresces a different color, allows each chromosome in a karyotype to be identified.



20 In situ hybridization is another technique for determining the chromosomal location of a gene. (a) FISH technique: in this case, the bound probe reveals sequences associated with the centromere. (b) SKY technique: 24 different probes, each specific for a different human chromosome and producing a different color, identify the different human chromosomes.

Mapping by DNA Sequencing

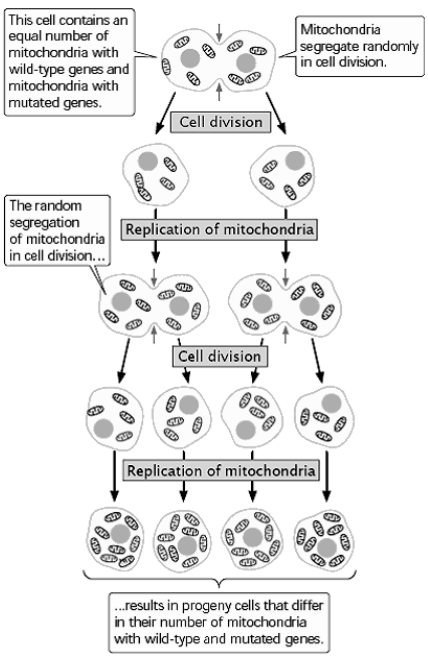
Another means of physically mapping genes is to determine the sequence of nucleotides in the DNA (DNA sequencing. With this technique, physical distances between genes are measured in numbers of base pairs. Continuous sequences can be determined for only relatively small fragments of DNA; so, after sequencing, some method is still required to map the individual fragments. This mapping is often done by using the traditional gene mapping that examines rates of crossing over between molecular markers located on the fragments. It can also be accomplished by generating a set of overlapping fragments, sequencing each fragment, and then aligning the fragments by using a computer program that identifies the overlap in the sequence of adjacent fragments. With these methods, complete physical maps of entire genomes have been produced .

E. Extra chromosomal inheritance: Inheritance of mitochondrial and chloroplast genes, maternal inheritance.

Cytoplasmic Inheritance

Mendel's principles of segregation and independent assortment are based on the assumption that genes are located on chromosomes in the nucleus of the cell. For the majority of genetic characteristics, this assumption is valid, and Mendel's principles allow us to predict the types of offspring that will be produced in a genetic cross. However, not all the genetic material of a cell is found in the nucleus; some characteristics are encoded by genes located in the cytoplasm.

These characteristics exhibit cytoplasmic inheritance. A few organelles, notably chloroplasts and mitochondria, contain DNA. Each human mitochondrion contains about 15,000 nucleotides of DNA, encoding 37 genes. Compared with that of nuclear DNA, which contains some 3 billion nucleotides encoding perhaps 35,000 genes, the amount of mitochondrial DNA (mtDNA) is very small; nevertheless, mitochondrial and chloroplast genes encode some important characteristics.



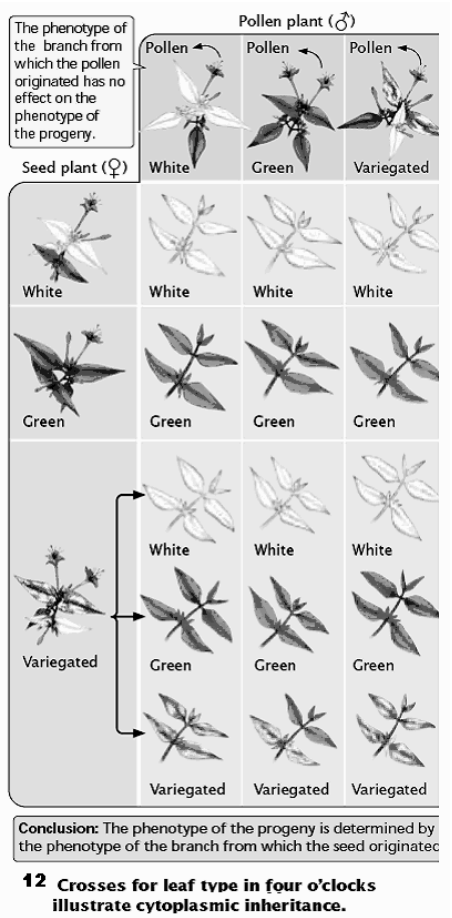
11 Cytoplasmically inherited characteristics frequently exhibit extensive phenotypic variation because cells and individual offspring contain various proportions of cytoplasmic genes.

Cytoplasmic inheritance differs from the inheritance of characteristics encoded by nuclear genes in several important respects. A zygote inherits nuclear genes from both parents, but typically all of its cytoplasmic organelles, and thus all its cytoplasmic genes, come from only one of the gametes, usually the egg. Sperm generally contributes only a set of nuclear genes from the male parent. In a few organisms, cytoplasmic genes are inherited from the male parent, or from both parents; however, for most organisms, all the cytoplasm is inherited from the egg. In this case, cytoplasmically inherited maits are present in both males and females and are passed from mother to offspring, never from father to offspring.

Reciprocal crosses, therefore, give different results when cytoplasmic genes encode a trait. Cytoplasmically inherited characteristics frequently exhibit extensive phenotypic variation, because there is no mechanism analogous to mitosis or meiosis to ensure that cytoplasmic genes are evenly distributed in cell division. Thus, different cells and individuals will contain various proportions of cytoplasmic genes.

Consider mitochondrial genes. There are thousands of mitochondria in each cell, and each mitochondrion contains from 2 to 10 copies of mtDNA. Suppose that half of the mitochondria in a cell contain a normal wild-type copy of mt DNA and the other half contain a mutated copy (FIGURE 11).

In cell division, the mitochondria segregate into progeny cells at random. Just by chance, one cell may receive mostly mutated mtDNA and another cell may receive mostly wild-type mtDNA (see Figure 11). In this way, different progeny from the same mother and even cells within an individual offspring may vary in their phenotype. Traits encoded by chloroplast DNA (cpDNA) are similarly variable.



12 Crosses for leaf type in four o'clocks illustrate cytoplasmic inheritance.

In 1909, cytoplasmic inheritance was recognized by Carl Correns as one of the first exceptions to Mendel's principles. Correns, one of the biologists who rediscovered Mendel's work, studied the inheritance of leaf variegation in the four-o'clock plant, Mirabilis jalapa. Correns found that the leaves and shoots of one variety of four-o'clock were variegated, displaying a mixture of green and white splotches. He also noted that some branches of the variegated strain had all-green leaves; other branches had all white leaves. Each branch produced flowers; so Correns was able to cross flowers from variegated, green, and white branches in all combinations (FIGURE 12).

The seeds from green branches always gave rise to green progeny, no matter whether the pollen was from a green, white, or variegated branch. Similarly, flowers on white branches always produced white progeny. Flowers on the variegated branches gave rise to green, white, and variegated progeny, in no particular ratio.

Corren's crosses demonstrated cytoplasmic inheritance of variegation in the four-o'clocks. The phenotypes of the offspring were determined entirely by the maternal parent, never by the paternal parent (the source of the pollen). Furthermore, the production of all three phenotypes by flowers on variegated branches is consistent with the occurrence of cytoplasmic inheritance. Variegation in these plants is caused by a defective gene in the cpDNA, which results in a failure to produce the green pigment chlorophyll. Cells from green branches contain normal chloroplasts only, cells from white branches contain abnormal chloroplasts only, and cells from variegated branches contain a mixture of normal and abnormal chloroplasts. In the flowers from variegated branches, the random segregation of chloroplasts in the course of oogenesis produces some egg cells with normal cpDNA, which develop into green progeny; other egg cells with only abnormal cpDNA develop into white progeny; and, finally, still other egg cells with a mixture of normal and abnormal cpDNA develop into variegated progeny.

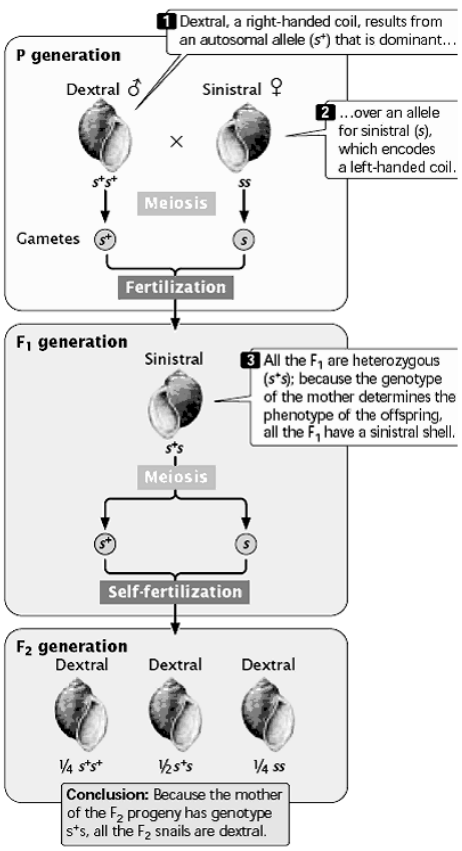
In recent years, a number of human diseases (mostly rare) that exhibit cytoplasmic inheritance have been identified. These disorders arise from mutations in mtDNA, most of which occur in genes coding for components of the electron-transport chain, which generates most of the ATP (adenosine triphosphate) in aerobic cellular respiration. One such disease is Leber hereditary optic neuropathy. Patients who have this disorder experience rapid loss of vision in both eyes, resulting from the death of cells in the optic nerve. Loss of vision typically occurs in early adulthood (usually between the ages of 20 and 24), but it can occur any time after adolescence. There is much clinical variability in the severity of the disease, even within the same family. Leber hereditary optic neuropathy exhibits maternal inheritance: the trait is always passed from mother to child.

Genetic Maternal Effect

A genetic phenomenon that is sometimes confused with cytoplasmic inheritance is genetic maternal effect, in which the phenotype of the offspring is determined by the genotype of the mother. In cytoplasmic inheritance, the genes for a characteristic are inherited from only one parent, usually the mother. In genetic maternal effect, the genes are inherited from both parents, but the offspring's phenotype is determined not by its own genotype but by the genotype of its mother.

Genetic maternal effect frequently arises when substances present in the cytoplasm of an egg (encoded by the mother's genes) are pivotal in early development. An excellent example is shell coiling of the snail Limnaea peregra. In most snails of this species, the shell coils to the right, which is termed dextral coiling. However, some snails possess a left-coiling shell, exhibiting sinistral coiling. The direction of coiling is determined by a pair of alleles; the allele for dextral (s_) is dominant over the allele for sinistral (s). However, the direction of coiling is determined not by that snail's own genotype, but by the genotype of its mother. The direction of coiling is affected by the way in which the cytoplasm divides soon after fertilization, which in turn is determined by a substance produced by the mother and passed to the offspring in the cytoplasm of the egg.

If a male homozygous for dextral alleles (s_s_) is crossed with a female homozygous for sinistral alleles (ss), all of the F1 are heterozygous (s_s) and have a sinistral shell, because the genotype of the mother (ss) codes for sinistral (FIGURE 13). If these F1 snails are self-fertilized, the genotypic ratio of the F2 is 1 s_s_:2 s_s:1 ss. The phenotype of all F2 snails will be dextral regardless of their genotypes, because the genotype of their mother (s_s) encodes a right coiling shell and determines their phenotype.



13 In genetic maternal effect, the genotype of the maternal parent determines the phenotype of the offspring. Shell coiling in snails is a trait that exhibits genetic maternal effect.

Notice that the phenotype of the progeny is not necessarily the same as the phenotype of the mother, because the progeny's phenotype is determined by the mother's genotype, not her phenotype. Neither the male parent's nor the offspring's own genotype has any role in the offspring's phenotype. A male does influence the phenotype of the F2 generation; by contributing to the genotypes of his daughters, he affects the phenotypes of their offspring. Genes that exhibit genetic maternal effect are therefore transmitted through males to future generations. In contrast, the genes that exhibit cytoplasmic inheritance are always transmitted through only one of the sexes (usually the female).

Genomic Imprinting

One of the basic tenets of Mendelian genetics is that the parental origin of a gene does not affect its expression—reciprocal crosses give identical results. We have seen that there are some genetic characteristics—those encoded by X-linked genes and cytoplasmic genes—for which reciprocal crosses do not give the same results. In these cases, males and females do not contribute the same genetic material to the offspring. With regard to autosomal genes, males and females contribute the same number of genes, and paternal and maternal genes have long been assumed to have equal effects. The results of recent studies, however, have identified several mammalian genes whose expression is significantly affected by their parental origin. This phenomenon, the differential expression of genetic material depending on whether it is inherited from the male or female parent, is called genomic imprinting.

Genomic imprinting has been observed in mice in which a particular gene has been artificially inserted into a mouse's DNA (to create a transgenic mouse). In these mice, the inserted gene is faithfully passed from generation to generation, but its expression may depend on which parent transmitted the gene. For example, when a transgenic male passes an imprinted gene to his offspring, they express the gene; but, when his daughter transmits the same gene to her offspring, they don't express it. In turn, her son's offspring express it, but her daughter's offspring don't. Both male and female offspring possess the gene for the trait; the key to whether the gene is expressed is the sex of the parent transmitting the gene. In the present example, the gene is expressed only when it is transmitted by a male parent. The reverse situation, expression of a trait when the gene is transmitted by the female parent, also occurs. Genomic imprinting has been implicated in several human disorders, including **Prader-Willi and Angelman syndromes**. Children with Prader-Willi syndrome have small hands and feet, short stature, poor sexual development, and mental retardation; they develop voracious appetites and frequently become obese. Many persons with Prader-Willi syndrome are missing a small region of chromosome 15 called q11–13. The deletion of this region is always inherited from the father in persons with Prader-Willi syndrome.

The deletions of q11–13 on chromosome 15 can also be inherited from the mother, but this inheritance results in a completely different set of symptoms, producing Angelman syndrome. Children with Angelman syndrome exhibit frequent laughter, uncontrolled muscle movement, a large mouth, and unusual seizures. The deletion of segment q11–13 from chromosome 15 has severe effects on the human phenotype, but the specific effects depend on which parent contributes the deletion. For normal development to take place, copies of segment q11–13 of chromosome 15 from both male and female parents are apparently required.

Several other human diseases also appear to exhibit genomic imprinting. Although the precise mechanism of this phenomenon is unknown, methylation of DNA—the addition of methyl (CH_3) groups to DNA nucleotides —is essential to the process of genomic imprinting, as demonstrated by the observation that mice deficient in DNA methylation do not exhibit imprinting. Some of the ways in which sex interacts with heredity are summarized in Table 4.

Table 4 Sex influences on heredity

Genetic Phenomenon	Phenotype Determined by
Sex-linked characteristic	genes located on the sex chromosome
Sex-influenced characteristic	genes on autosomal chromosomes that are more readily expressed in one sex
Sex-limited characteristic	autosomal genes whose expression is limited to one sex
Genetic maternal effect	nuclear genotype of the maternal parent
Cytoplasmic inheritance	cytoplasmic genes, which are usually inherited entirely from only one parent
Genomic imprinting	genes whose expression is affected by the sex of the transmitting parent

Anticipation

Another genetic phenomenon that is not explained by Mendel's principles is anticipation, in which a genetic trait becomes more strongly expressed or is expressed at an earlier age as it is passed from generation to generation. In the early 1900s, several physicians observed that patients with moderate to severe myotonic dystrophy—an autosomal dominant muscle disorder—frequently had ancestors who were only mildly affected by the disease. These observations led to the concept of anticipation. However, the concept quickly fell out of favor with geneticists because there was no obvious mechanism to explain it; traditional genetics held that genes are passed unaltered from parents to offspring. Geneticists tended to attribute anticipation to observational bias.

The results of recent research have reestablished anticipation as a legitimate genetic phenomenon. The mutation causing myotonic dystrophy consists of an unstable region of DNA that can increase or decrease in size as the gene is passed from generation to generation, much like the gene that causes Huntington disease. The age of onset and the severity of the disease are correlated with the size of the unstable region; an increase in the size of the region through generations produces anticipation. The phenomenon has now been implicated in several genetic diseases.

Interaction Between Genes and Environment

We have learned that each phenotype is the result of a genotype developing within a specific environment; the genotype sets the potential for development, but how the phenotype actually develops within the limits imposed by the genotype depends on environmental effects. Stated another way, each genotype may produce several different phenotypes, depending on the environmental conditions in which development occurs. For example, genotype GG may produce a plant that is 10 cm high when raised at 20°C, but the same genotype may produce a plant that is 18 cm high when raised at 25°C. The range of phenotypes produced by a genotype in different environments (in this case, plant height) is called the norm of reaction. For most of the characteristics discussed so far, the effect of the environment on the phenotype has been slight. Mendel's peas with genotype yy, for example, developed yellow endosperm regardless of the environment in which they were raised. Similarly, persons with genotype I^AI^A have the A antigen on their red blood cells regardless of their diet, socioeconomic status, or family environment. For other phenotypes, however, environmental effects play a more important role.

Environmental Effects on Gene Expression

The expression of some genotypes is critically dependent on the presence of a specific environment. For example, the himalayan allele in rabbits produces dark fur at the extremities of the body—on the nose, ears, and feet. The dark pigment develops, however, only when the rabbit is reared at 25°C or less; if a Himalayan rabbit is reared at 30°C, no dark patches develop. The expression of the himalayan allele is thus temperature dependent—an enzyme necessary for the production of dark pigment is inactivated at higher temperatures. The pigment is normally restricted to the nose, feet, and ears of Himalayan rabbits because the animal's core body temperature is normally above 25°C and the enzyme is functional only in the cells of the relatively cool extremities. The himalayan allele is an example of a temperature-sensitive allele, an allele whose product is functional only at certain temperatures.

Some types of albinism in plants are temperature dependent. In barley, an autosomal recessive allele inhibits chlorophyll production, producing albinism when the plant is grown below 7°C. At temperatures above 18°C, a plant homozygous for the albino allele develops normal chlorophyll and is green. Similarly, among Drosophila melanogaster homozygous for the autosomal mutation vestigial, greatly reduced wings develop at 25°C, but wings near normal size develop at higher temperatures.

Environmental factors also play an important role in the expression of a number of human genetic diseases. Glucose-6-phosphate dehydrogenase is an enzyme taking part in supplying energy to the cell. In humans, there are a number of genetic variants of glucose-6-phosphate dehydrogenase, some of which destroy red blood cells when the body is stressed by infection or by the ingestion of certain drugs or foods. The symptoms of the genetic disease appear only in the presence of these specific environmental factors. Another genetic disease, phenylketonuria (PKU), is due to an autosomal recessive allele that causes mental retardation. The disorder arises from a defect in an enzyme that normally metabolizes the amino acid phenylalanine. When this enzyme is defective, phenylalanine is not metabolized, and its buildup causes brain damage in children. A simple environmental change, putting an affected child on a lowphenylalanine diet, prevents retardation.

These examples illustrate the point that genes and their products do not act in isolation; rather, they frequently interact with environmental factors. Occasionally, environmental factors alone can produce a phenotype that is the same as the phenotype produced by a genotype; this phenotype is called a phenocopy. In fruit flies, for example, the autosomal recessive mutation eyeless produces greatly reduced eyes. The eyeless phenotype can also be produced by exposing the larvae of normal flies to sodium metaborate.

The Inheritance of Continuous Characteristics

So far, we've dealt primarily with characteristics that have only a few distinct phenotypes. In Mendel's peas, for example, the seeds were either smooth or wrinkled, yellow or green; the coats of dogs were black, brown, or yellow; blood types were of four distinct types, A, B, AB, or O. Characteristics such as these, which have a few easily distinguished phenotypes, are called discontinuous characteristics.

Not all characteristics exhibit discontinuous phenotypes. Human height is an example of such a character; people do not come in just a few distinct heights but, rather, display a continuum of heights. Indeed, there are so many possible phenotypes of human height that we must use a measurement to describe a person's height. Characteristics that exhibit a continuous distribution of phenotypes are termed continuous characteristics. Because such characteristics have many possible phenotypes and must be described in quantitative terms, continuous characteristics are also called quantitative characteristics.

F. Microbial genetics: Methods of genetic transfers – transformation, conjugation, transduction and sex-duction, mapping genes by interrupted mating, fine structure analysis of genes.

Gene Transfer in Bacteria

For many years, bacteria were thought to reproduce only by simple binary fission, in which one cell splits into two identical cells without any exchange or recombination of genetic material. In 1946, Joshua Lederberg and Edward Tatum demonstrated that bacteria can transfer and recombine genetic information. This finding paved the way for the use of bacteria as model genetic organisms. Bacteria exchange genetic material by three different mechanisms,

1. **Conjugation** (FIGURE 1a) is the direct transfer of genetic material from one bacterium to another. In conjugation, two bacteria lie close together and a connection forms between them. A plasmid or a part of the bacterial chromosome passes from one cell (the donor) to the other (the recipient). Subsequent to conjugation, crossing over takes place between homologous sequences in the transferred DNA and the chromosome of the recipient cell. In conjugation, DNA is transferred only from donor to recipient, with no reciprocal exchange of genetic material.

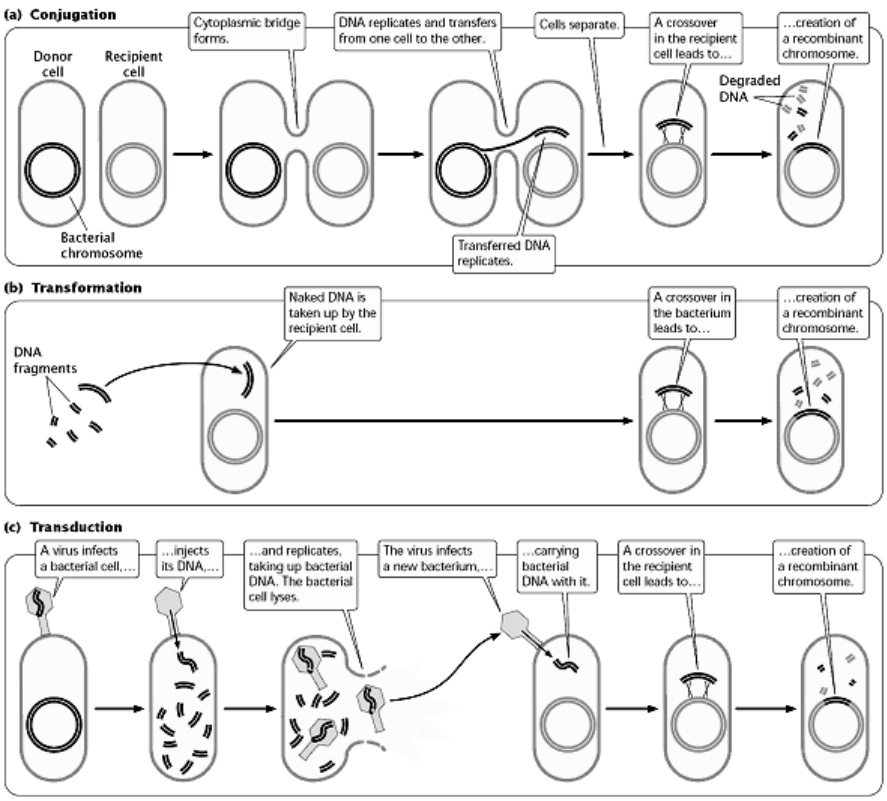
2. In **transformation** (FIGURE 1b),DNA in the medium surrounding a bacterium is taken up. After transformation, recombination may take place between the introduced genes and those of the bacterial chromosome.

3. In **transduction** (FIGURE 1c), bacterial viruses (bacteriophages) carry DNA from one bacterium to another. Once inside the bacterium, the newly introduced DNA may undergo recombination with the bacterial chromosome.

Not all bacterial species exhibit all three types of genetic transfer. Conjugation is more frequent for some bacteria than for others. Transformation takes place to a limited extent in many bacteria, but laboratory techniques have been developed that increase the rate of DNA uptake. Most bacteriophages have a limited host range; so transduction is normally between bacteria of the same or closely related species only.

These processes of genetic exchange in bacteria differ from the sexual reproduction of diploid eukaryotes in two

important ways. First, DNA exchange and reproduction are not coupled in bacteria. Second, donated genetic material that is not recombined into the host DNA is usually degraded and so the recipient cell remains haploid. Each type of genetic transfer can be used to map genes, as will be discussed in the following sections.



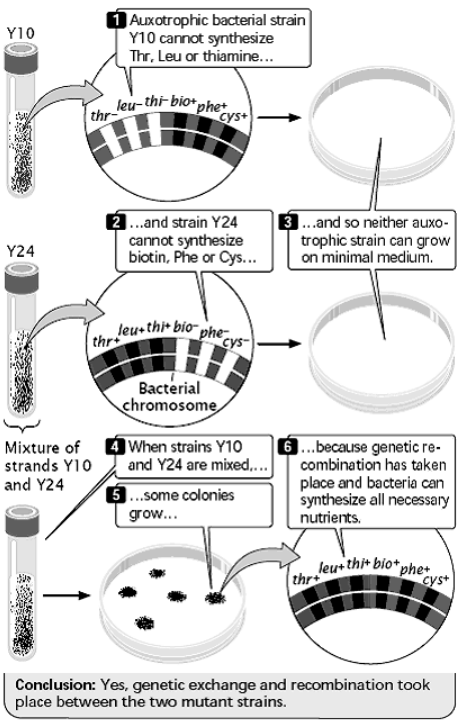
1 Conjugation, transformation, and transduction are three processes of gene transfer in bacteria. All three processes require transferred DNA to undergo recombination with the bacterial chromosome for the transferred DNA to be stably inherited.

Conjugation

In the course of their research, Lederberg and Tatum studied strains of E. coli possessing auxotrophic mutations.

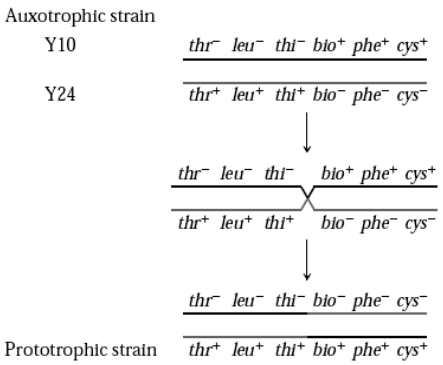
The Y10 strain required the amino acids threonine (and genotypically was thr^-) and leucine (leu^-) and the vitamin thiamine (thi^-) for growth but did not require the vitamin biotin (bio^+) or the amino acids phenylalanine (phe^+) and cysteine (cys^+); the genotype of this strain can be written as: thr^- leu^- thi^- bio^+ phe^+ cys^+. The Y24 strain required biotin, phenylalanine, and cysteine in its medium, but it did not require threonine, leucine, or thiamine; its genotype was: thr^+ leu^+ thi^+ bio^- phe^- cys^-. In one experiment, Lederberg and Tatum mixed Y10 and Y24 bacteria together and plated them on minimal medium (FIGURE 2). Each strain was also plated separately on minimal medium.

Alone, neither Y10 nor Y24 grew on minimal medium. Strain Y10 was unable to grow, because it required threonine, leucine, and thiamine, which were absent in the minimal medium; strain Y24 was unable to grow, because it required biotin, phenylalanine, and cysteine, which also were absent from the minimal medium. When Lederberg and Tatum mixed the two strains, however, a few colonies did grow on the minimal medium. These prototrophic bacteria must have had genotype thr^+ leu^+ thi^+ bio^+ phe^+ cys^+.

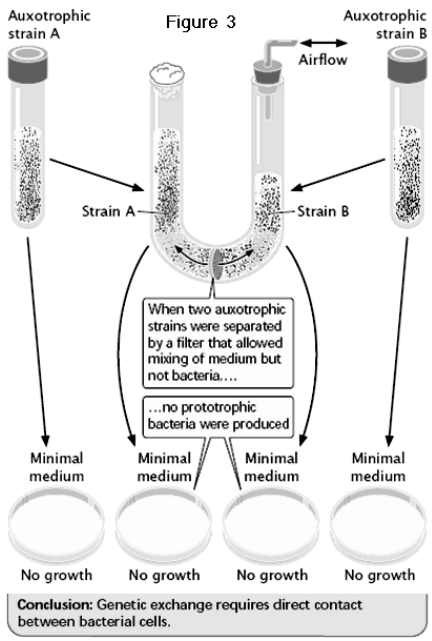


2 **Lederberg and Tatum's experiment demonstrated that bacteria undergo genetic exchange.**

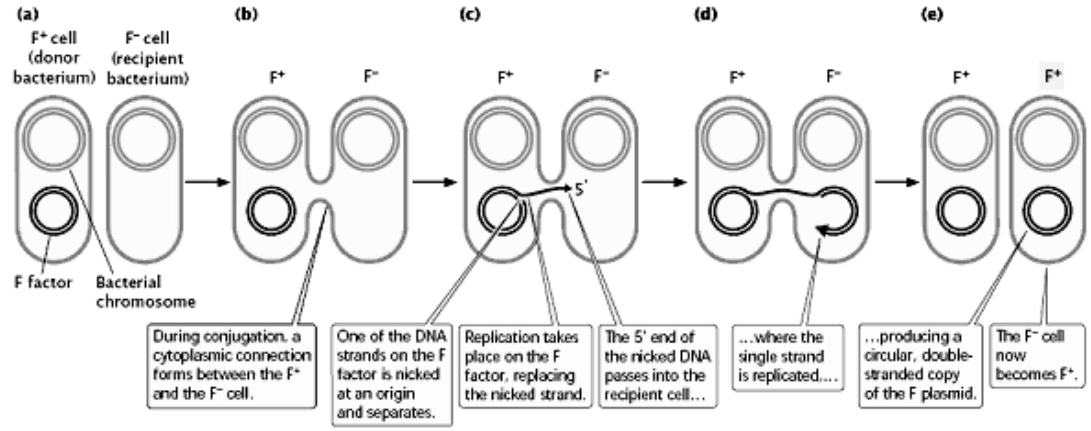
Where had they come from? If mutations were responsible for the prototrophic colonies, then some colonies should also have grown on the plates containing Y10 or Y24 alone, but no bacteria grew on these plates. Multiple simultaneous mutations ($thr^- \rightarrow thr^+$, $leu^- \rightarrow leu^+$, and $thi^- \rightarrow thi^+$ in strain Y10 or $bio^- \rightarrow bio^+$, $phe^- \rightarrow phe^+$, and $cys^- \rightarrow cys^+$ in strain Y24) would have been required for either strain to become prototrophic by mutation, which was very improbable. Lederberg and Tatum concluded that some type of genetic transfer and recombination had taken place:



What they did not know was how it had taken place. To study this problem, Bernard Davis constructed a U-shaped tube (FIGURE 3) that was divided into two compartments by a filter having fine pores. This filter allowed liquid medium to pass from one side of the tube to the other, but the pores of the filter were too small to allow passage of bacteria. Two auxotrophic strains of bacteria were placed on opposite sides of the filter, and suction was applied alternately to the ends of the U-tube, causing the medium to flow back and forth between the two compartments. Despite hours of incubation in the U-tube, bacteria plated out on minimal medium did not grow; there had been no genetic exchange between the strains. The exchange of bacterial genes clearly required direct contact between the bacterial cells. This type of genetic exchange entailing cell-to-cell contact in bacteria is called conjugation.



F^+ and F^- cells In most bacteria, conjugation depends on a fertility (F) factor that is present in the donor cell and absent in the recipient cell. Cells that contain F are referred to as F^+, and cells lacking F are F^-. The F factor contains an origin of

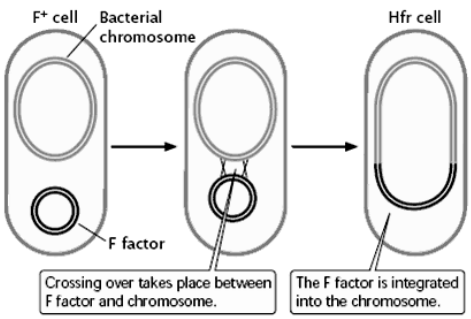


4 The F factor is transferred during conjugation between an F+ and F− cell.

replication and a number of genes required for conjugation. For example, some of these genes encode sex pili (singular, pilus), slender extensions of the cell membrane. A cell containing F produces the sex pili, which makes contact with a receptor on an F^- cell (FIGURE 2) and pulls the two cells together. DNA is then transferred from the F^+ cell to the F^- cell. Conjugation can take place only between a cell that possesses F and a cell that lacks F.

Davis's U-tube experiment.

In most cases, the only genes transferred during conjugation between an F^+ and F^- cell are those on the F factor (FIGURE 4a and b). Transfer is initiated when one of the DNA strands on the F factor is nicked at an origin (oriT). One end of the nicked DNA separates from the circle and passes into the recipient cell (FIGURE 4c). Replication takes place on the nicked strand, proceeding around the circular plasmid and replacing the transferred strand (FIGURE 4d). Because the plasmid in the F^+ cell is always nicked at the oriT site, this site always enters the recipient cell first, followed by the rest of the plasmid. Thus, the transfer of genetic material has a defined direction. Once inside the recipient cell, the single strand is replicated, producing a circular, double-stranded copy of the F plasmid (FIGURE 4e). If the entire F factor is transferred to the recipient F^+ cell, that cell becomes an F^- cell. Hfr cells Conjugation transfers genetic material in the F plasmid from F^+ to F^- cells but does not account for the transfer of chromosomal genes observed by Lederberg and Tatum.

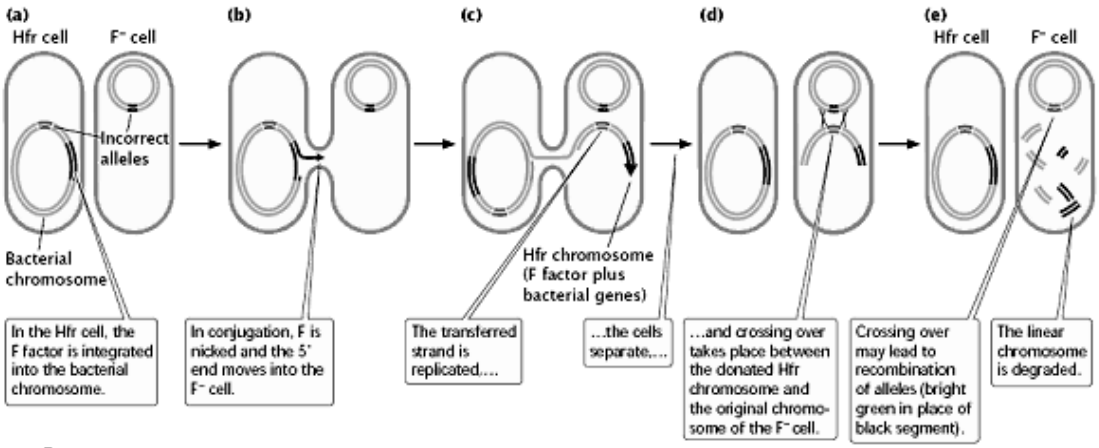


5 The F factor is integrated into the bacterial chromosome in an Hfr cell.

In Hfr (high-frequency) strains, the F factor is integrated into the bacterial chromosome (FIGURE 5). Hfr cells behave as F^+ cells, forming sex pili and undergoing conjugation with F^- cells.

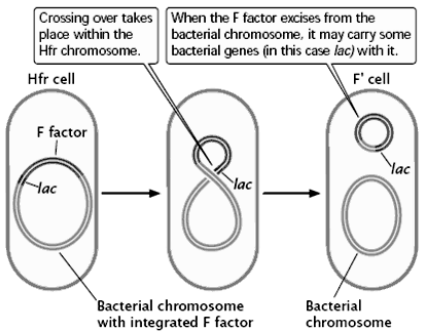
In conjugation between Hfr and F^- cells (FIGURE 6a), the integrated F factor is nicked, and the end of the nicked strand moves into the F^- cell (FIGURE 6b), just as it does in conjugation between F^+ and F^- cells. In the Hfr cells, the F factor is linked to the bacterial chromosome, so the chromosome follows it into the recipient cell. How much of the bacterial chromosome is transferred depends on the length of time that the two cells remain in conjugation.

Once inside the recipient cell, the donor DNA strand is replicated (FIGURE 6c), and crossing over between it and the original chromosome of the F^- cell (FIGURE 6d) may take place. This gene transfer between Hfr and F^- cells is how the recombinant prototrophic cells observed by Lederberg and Tatum were produced. When crossing over has taken place in the recipient cell, the donated chromosome is degraded, and the recombinant recipient chromosome remains (FIGURE 6e) to be replicated and passed to later generations by binary fission. In a mating of Hfr x F^-, the F^- cell almost never becomes F^+ or Hfr, because the F factor is nicked in the middle during the initiation of strand transfer, placing part of F at the beginning and part at the end of the strand to be transferred. To become F^+ or Hfr, the recipient cell must receive the entire F factor, requiring that the entire bacterial chromosome is transferred. This event happens rarely, because most conjugating cells break apart before the entire chromosome has been transferred. The F plasmid in F^+ cells integrates into the bacterial chromosome, causing an F^+ cell to become Hfr, at a frequency of only about 1/10,000. This low frequency accounts for the low rate of recombination observed by Lederberg and Tatum in their F^+ cells. The F factor is excised from the bacterial chromosome at a similarly low rate, causing a few Hfr cells to become F^+.



6 Bacterial genes may be transferred from an Hfr cell to an F⁻ cell in conjugation.

F' cells: When an F factor does excise from the bacterial chromosome, a small amount of the bacterial chromosome may be removed with it, and these chromosomal genes will then be carried with the F plasmid (FIGURE 7). Cells containing an F plasmid with some bacterial genes are called F prime (F'). For example, if an F factor integrates into a chromosome adjacent to the chromosome's lac operon, the F factor may pick up lac genes when it excises, becoming F'lac. F' cells can conjugate with F⁻ cells, given that they possess the F plasmid with all the genetic information necessary for conjugation and gene transfer.



7 An Hfr cell may be converted into an F' cell when the F factor excises from the bacterial chromosome and carries bacterial genes with it.

Characteristics of different mating types of E. coli (cells with different types of F) are summarized in Table 2.

Table 2 Characteristics of *E. coli* cells with different types of F factor

Type	F Factor Characteristics	Role in Conjugation
F⁺	Present as separate circular DNA	Donor
F⁻	Absent	Recipient
Hfr	Present, integrated into bacterial chromosome	High-frequency donor
F'	Present as separate circular DNA, carrying some bacterial genes	Donor

During conjugation between an F'lac cell and an F⁻ cell, the F plasmid is transferred to the F⁻ cell, which means that any genes on the F plasmid, including those from the bacterial chromosome, may be transferred to F⁻ recipient cells. This process is called sexduction. It produces partial diploids, or merozygotes, which are cells with two copies of some genes, one on the bacterial chromosome and one on the newly introduced F plasmid. The outcomes of conjugation between different mating types of E. coli are summarized in Table 3.

Table 3 Results of conjugation between cells with different F factors

Conjugating Cells	Cell Types Present After Conjugation
F⁺ × F⁻	Two F⁺ cells (F⁻ cell becomes F⁺)
Hfr × F⁻	One F⁺ cell and one F⁻ (no change)*
F' × F⁻	Two F' cells (F⁻ cell becomes F')

*Rarely, the F⁻ cell becomes F⁺ in an Hfr × F⁻ conjugation if the entire chromosome is transferred during conjugation.

Mapping bacterial genes with interrupted conjugation The transfer of DNA that takes place during conjugation between Hfr and F⁻ cells allows bacterial genes to be mapped. During conjugation, the chromosome of the Hfr cell is transferred to the F⁻ cell. Transfer of the entire E. coli chromosome requires about 100 minutes; if conjugation is interrupted before 100 minutes have elapsed, only part of the chromosome will pass into the F⁻ cell and have an opportunity to recombine with the recipient chromosome. Chromosome transfer always begins within the integrated F factor and proceeds in a continuous direction; so genes are transferred according to their sequence on the chromosome. The time required for individual genes to be transferred indicates their relative positions on the chromosome. In most genetic maps, distances are expressed as percent recombination; but, in bacterial maps constructed with interrupted conjugation, the basic unit of distance is a minute.

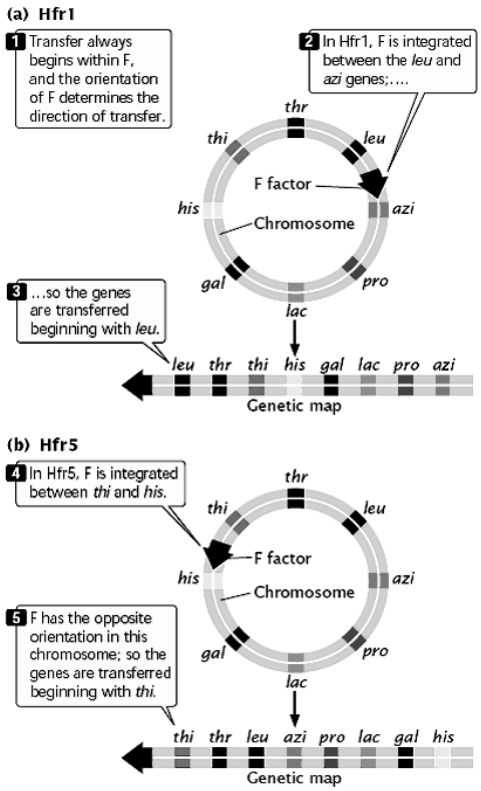
Directional transfer and mapping Different Hfr strains have the F factor integrated into the bacterial chromosome at different sites and in different orientations. Gene transfer always begins within F, and the orientation and position of F determine the direction and starting point of gene transfer. In FIGURE 8a, strain Hfr1 has F integrated between leu and

azi; the orientation of F at this site dictates that gene transfer will proceed in a counterclockwise direction around the circular chromosome. Genes from this strain will be transferred in the order of:

$$\leftarrow leu-thr-thi-his-gal-lac-pro-azi$$

Strain Hfr5 has F integrated between the thi and his genes (FIGURE 8b) and in the opposite orientation. Here gene transfer will proceed in a clockwise direction:

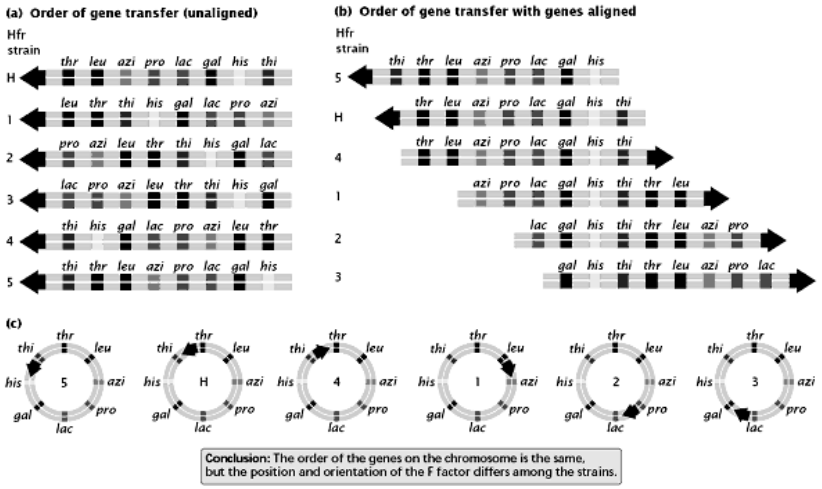
$$\leftarrow thi-thr-leu-azi-pro-lac-gal-his$$



8 **The orientation of the F factor in an Hfr strain determines the direction of gene transfer.** Arrowheads indicate the origin and direction of transfer.

Although the starting point and direction of transfer may differ between two strains, the relative distance in time between any two pairs of genes is constant.

Notice that the order of gene transfer is not the same for different Hfr strains (FIGURE 9a). For example, azi is transferred just after leu in strain HfrH, but long after leu in strain Hfr1. Aligning the sequences (FIGURE 9b) shows that the two genes on either side of azi are always the same: leu and pro. That they are the same makes sense when one recognizes that the bacterial chromosome is circular and the starting point of transfer varies from strain to strain. These data provided the first evidence that the bacterial chromosome is circular (FIGURE 9c).



9 **The order of gene transfer in a series of different Hfr strains indicates that the *E. coli* chromosome is circular.**

Natural Gene Transfer and Antibiotic Resistance

Many pathogenic bacteria have developed resistance to antibiotics, particularly in environments where antibiotics are routinely used, such as hospitals and fish farms. The continual presence of antibiotics in these environments selects for resistant bacteria, which reduces the effectiveness of antibiotic treatment for medically important infections. Antibiotic resistance in bacteria frequently results from the action of genes located on R plasmids, small circular plasmids that can be transferred by conjugation. R plasmids have evolved in the past 50 years (since the beginning of widespread use of antibiotics), and some convey resistance to several antibiotics simultaneously. Ironic but plausible sources of some of the resistance genes found in R plasmids are the microbes that produce antibiotics in the first place. The results of recent studies demonstrate that R plasmids and their resistance genes are transferred among bacteria in a variety of natural environments. In one study, plasmids carrying genes for resistance to multiple antibiotics were transferred from a cow udder infected with E. coli to a human strain of E. coli on a hand towel: a farmer wiping his hands after milking an infected cow might unwittingly transfer antibiotic resistance from bovine- to human-inhabiting microbes. Conjugation taking place in minced meat on a cutting board allowed R plasmids to be passed from porcine (pig) to human E. coli. The transfer of R plasmids also occurs in sewage, soil, lake water, and marine sediments.

Perhaps most significantly, the transfer of R plasmids is not restricted to bacteria of the same or even related species. R plasmids with multiple antibiotic resistances have been transferred in marine waters from E. coli and other human inhabiting bacteria (in sewage) to the fish bacterium *Aeromona salmonicida* and then back to E. coli through raw salmon chopped on a cutting board. These results indicate that R plasmids can spread easily through the environment, passing among related and unrelated bacteria in a variety of common situations. That they can do so underscores both the importance of limiting antibiotic use to treating medically important infections and the importance of hygiene in everyday life.

Transformation in Bacteria

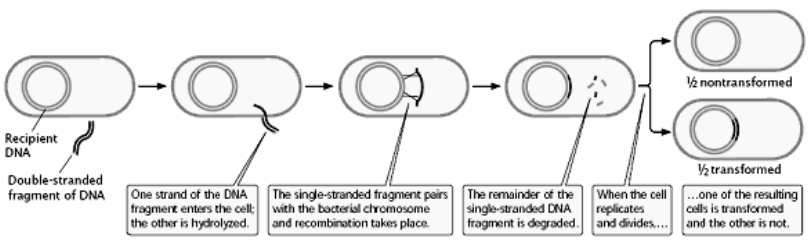
A second way that DNA can be transferred between bacteria is through transformation (see Figure 1b). Transformation played an important role in the initial identification of DNA as the genetic material. Transformation requires both the uptake of DNA from the surrounding medium and its incorporation into the bacterial chromosome or a plasmid. It may occur naturally when dead bacteria break up and release DNA fragments into the environment. In soil and marine environments, this means may be an important route of genetic exchange for some bacteria.

Cells that take up DNA are said to be competent. Some species of bacteria take up DNA more easily than do others; competence is influenced by growth stage, the concentration of available DNA, and the composition of the medium.

The uptake of DNA fragments into a competent bacterial cell appears to be a random process. The DNA need not even be bacterial: virtually any type of DNA (bacterial or otherwise) can be transferred to competent cells under the appropriate conditions.

As a DNA fragment enters the cell in the course of transformation (FIGURE 10), one of the strands is hydrolyzed, whereas the other strand associates with proteins as it moves across the membrane. Once inside the cell, this single strand may pair with a homologous region and become integrated into the bacterial chromosome. This integration requires two crossover events, after which the remaining single-stranded DNA is degraded by bacterial enzymes.

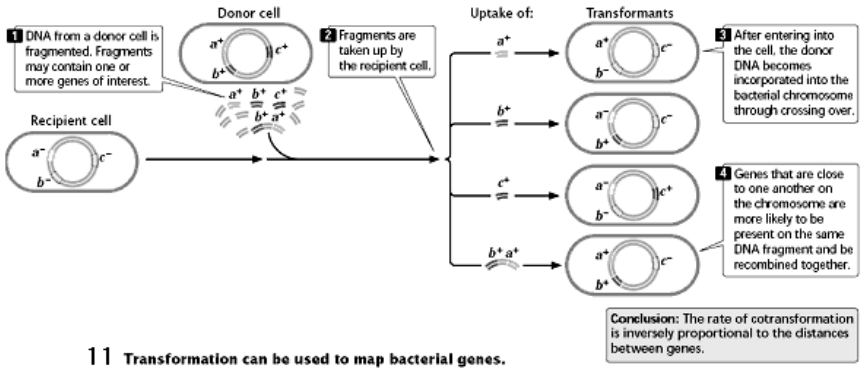
Bacterial geneticists have developed techniques to increase



10 Genes can be transferred between bacteria through transformation.

the frequency of transformation in the laboratory in order to introduce particular DNA fragments into cells. They have developed strains of bacteria that are more competent than wild-type cells. Treatment with calcium chloride, heat shock, or an electrical field makes bacterial membranes more porous and permeable to DNA, and the efficiency of transformation can also be increased by using high concentrations of DNA. These techniques make it possible to transform bacteria such as E. coli, which are not naturally competent.

Transformation, like conjugation, is used to map bacterial genes, especially in those species that do not undergo conjugation or transduction (see Figure 8.9a and c). Transformation mapping requires two strains of bacteria that differ in several genetic traits; for example, the recipient strain might be $a^-b^-c^-$(auxotrophic for three nutrients), with the donor cell being prototrophic with alleles $a^+b^+c^+$. DNA from the donor strain is isolated and purified. The recipient strain is treated to increase competency, and DNA from the donor strain is added to the medium. Fragments of the donor DNA enter the recipient cells and undergo recombination with homologous DNA sequences on the bacterial chromosome. Cells that receive genetic material through transformation are called transformants.



11 Transformation can be used to map bacterial genes.

Genes can be mapped by observing the rate at which two or more genes are transferred together (cotransformed) in transformation. When the DNA is fragmented during isolation, genes that are physically close on the chromosome are more likely to be present on the same DNA fragment and transferred together, as shown for genes a^+ and b^+ in FIGURE 11.

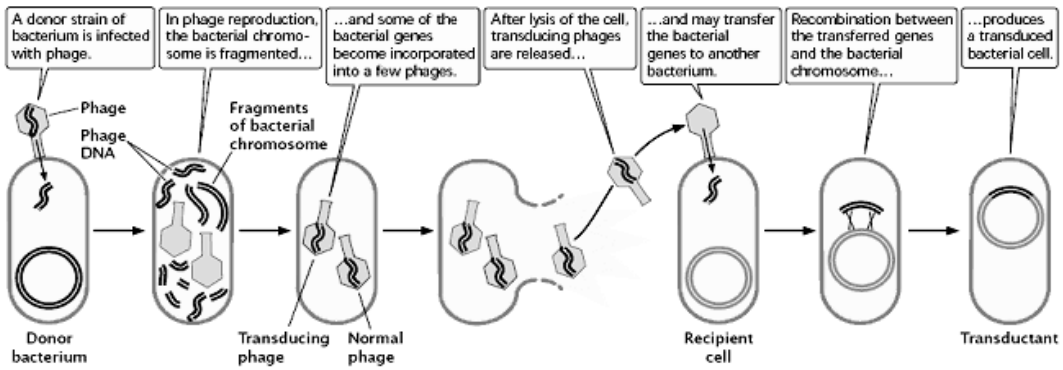
Genes that are far apart are unlikely to be present on the same DNA fragment and rarely will be transferred together. Once inside the cell, DNA becomes incorporated into the bacterial chromosome through recombination. If two genes are close together on the same fragment, any two crossovers are likely to occur on either side of the two genes, allowing both to become part of the recipient chromosome. If the two genes are far apart, there may be one crossover between them, allowing one gene but not the other to recombine with the bacterial chromosome. Thus, two genes are more likely to be transferred together when they are close together on the chromosome, and genes located far apart are rarely co-transformed. Therefore, the frequency of co-transformation can be used to map bacterial genes. If genes a and b are frequently co-transformed, and genes b and c are frequently co-transformed, but genes a and c are rarely co-transformed, then gene b must be between a and c—the gene order is a b c.

Transduction: Using Phages to Map Bacterial Genes

In the discussion of bacterial genetics, we identified three mechanisms of gene transfer: conjugation, transformation, and transduction (see Figure 1). Let's take a closer look at transduction, in which genes are transferred between bacteria by viruses. In generalized transduction, any gene may be transferred. In specialized transduction, only a few genes are transferred.

Generalized transduction Joshua Lederberg and Norton Zinder discovered generalized transduction in 1952. They were trying to produce recombination in the bacterium Salmonella typhimurium by conjugation. They mixed a strain of S. typhimurium that was phe$^+$ trp$^+$ tyr$^+$ met$^-$ his$^-$ with a strain that was phe$^-$ trp$^-$ tyr$^-$ met$^+$ his$^+$ and plated them on minimal medium.

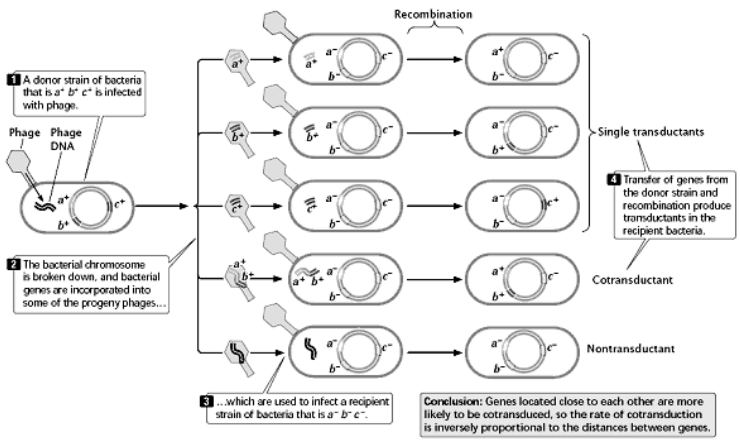
A few prototrophic recombinants (phe$^+$ trp$^+$ tyr$^+$ met$^+$ his$^+$) appeared, suggesting that conjugation had taken place. However, when they tested the two strains in a U-shaped tube similar to the one used by Davis, some phe$^+$ trp$^+$ tyr$^+$ met$^+$ his$^+$ prototrophs were obtained on one side of the tube. This apparatus separated the two strains by a filter with pores too small for the passage of bacteria; so how were genes being transferred between bacteria in the absence of conjugation? The results of subsequent studies revealed that the agent of transfer was a bacteriophage. In the lytic cycle of phage reproduction, the bacterial chromosome is broken into random fragments (FIGURE 12). For some types of bacteriophage, a piece of the bacterial chromosome occasionally gets packaged into a phage coat instead of phage DNA; these phage particles are called transducing phages. The transducing phage infects a new cell, releasing the bacterial DNA, and the introduced genes may then become integrated into the bacterial chromosome by a double crossover. Bacterial genes can, by this process, be moved from one bacterial strain to another, producing recombinant bacteria called transductants.



12 **Genes can be transferred from one bacterium to another through generalized transduction.**

Not all phages are capable of transduction, a rare event that requires (1) that the phage degrade the bacterial chromosome; (2) that the process of packaging DNA into the phage protein not be specific for phage DNA; and (3) that the bacterial genes transferred by the virus recombine with the chromosome in the recipient cell.

Because of the limited size of a phage particle, only about 1% of the bacterial chromosome can be transduced. Only genes located close together on the bacterial chromosome will be transferred together (cotransduced). The overall rate of transduction ranges from only about 1 in 100,000 to 1 in 1,000,000. Because the chance of a cell being transduced by two separate phages is exceedingly small, any cotransduced genes are usually located close together on the bacterial chromosome. Thus, rates of cotransduction, like rates of cotransformation, give an indication of the physical distances between genes on a bacterial chromosome. To map genes by using transduction, two bacterial strains with different alleles at several loci are used (FIGURE 13). The donor strain is infected with phages, which reproduce within the cell. When the phages have lysed the donor cells, a suspension of the progeny phage is mixed with a recipient strain of bacteria, which are then plated on several different kinds of media to determine the phenotypes of the transducing progeny phages.

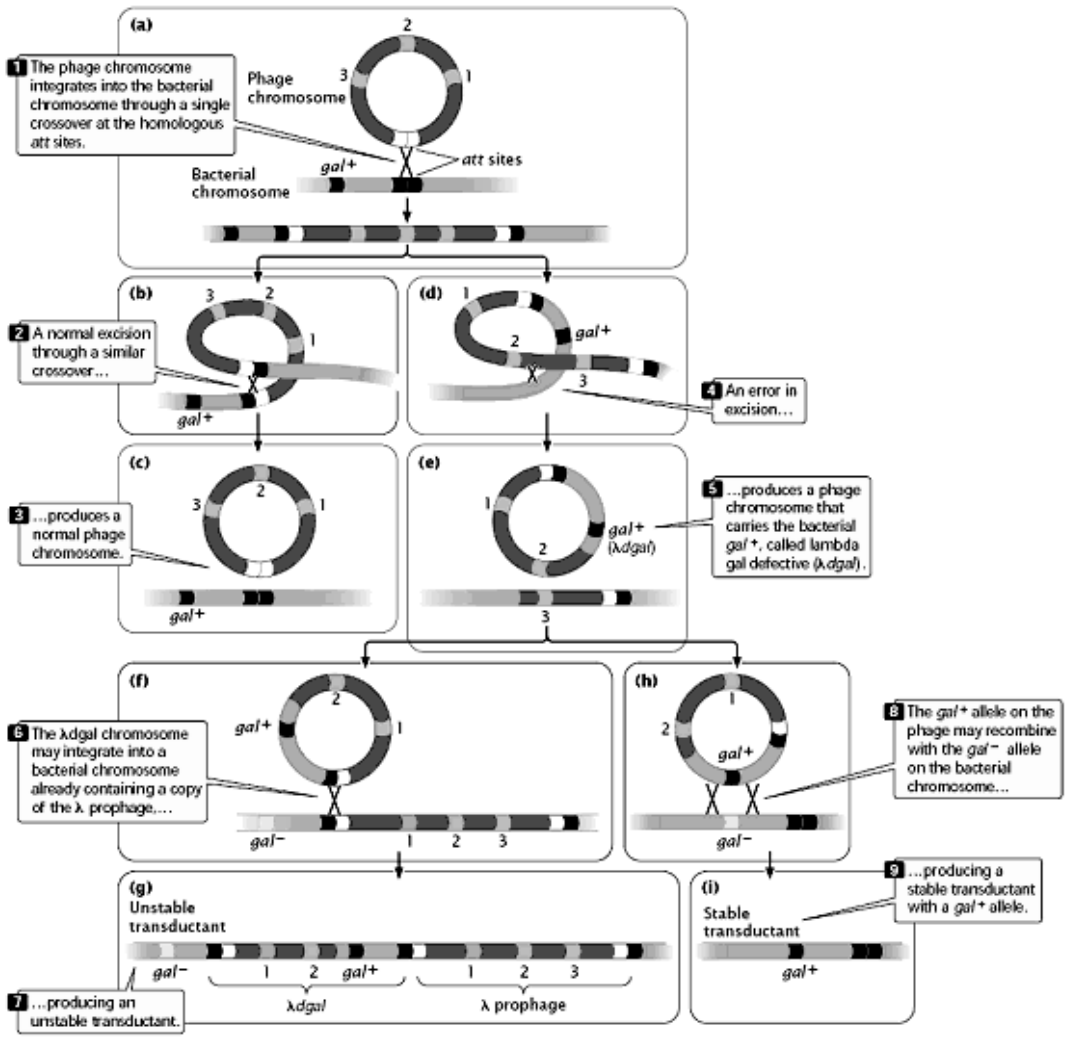


13 **Generalized transduction can be used to map genes.**

Specialized transduction Like generalized transduction, specialized transduction requires gene transfer from one bacterium to another through phages, but here only genes near particular sites on the bacterial chromosome are transferred.

This process requires lysogenic bacteriophages. The prophage may imperfectly excise from the bacterial chromosome, carrying with it a small part of the bacterial DNA adjacent to the site of prophage integration. A phage carrying this DNA will then inject it into another bacterial cell in the next round of infection. This process resembles the situation in F'cells, where the F plasmid carries genes from one bacterium into another (see Figure 8.16). One of the best-studied examples of specialized transduction is in bacteriophage lambda (λ), which integrates into the E. coli chromosome at the attachment (att) site. The phage DNA contains a site similar to the att site; a single crossover integrates the phage DNA into the bacterial chromosome

(FIGURE 14a). The λ prophage is excised through a similar crossover that reverses the process (FIGURE 14b and c). An error in excision may cause genes on either side of the bacterial att site to be excised along with some of the phage DNA (FIGURE 14d and e). In E. coli, these genes are usually the gal (galactose fermentation) and bio (biotin biosynthesis) genes. When a transducing phage carrying the gal gene infects another bacterium, the gene may integrate into the bacterial chromosome along with the prophage (FIGURE 14f), giving the bacterial chromosome two copies of the gal gene (FIGURE 14g). These transductants are unstable, because the prophage DNA may excise from the chromosome, carrying the introduced gene with it. Stable transductants are produced when the gal gene in the phage is exchanged for the gal gene in the chromosome through a double crossover .



14

Bacteria can exchange genes through specialized transduction. Segments 1, 2, and 3 represent genes on the phage chromosome.

G. Human genetics: Pedigree analysis, lod score for linkage testing, karyotypes, genetic disorders.

The Study of Human Genetic Characteristics

Humans are the best and the worst of all organisms for genetic study. On the one hand, we know more about human anatomy, physiology, and biochemistry than we know about most other organisms; for many families, we have detailed records extending back many generations; and the medical implications of genetic knowledge of humans provide tremendous incentive for genetic studies. On the other hand, the study of human genetic characteristics presents some major obstacles.

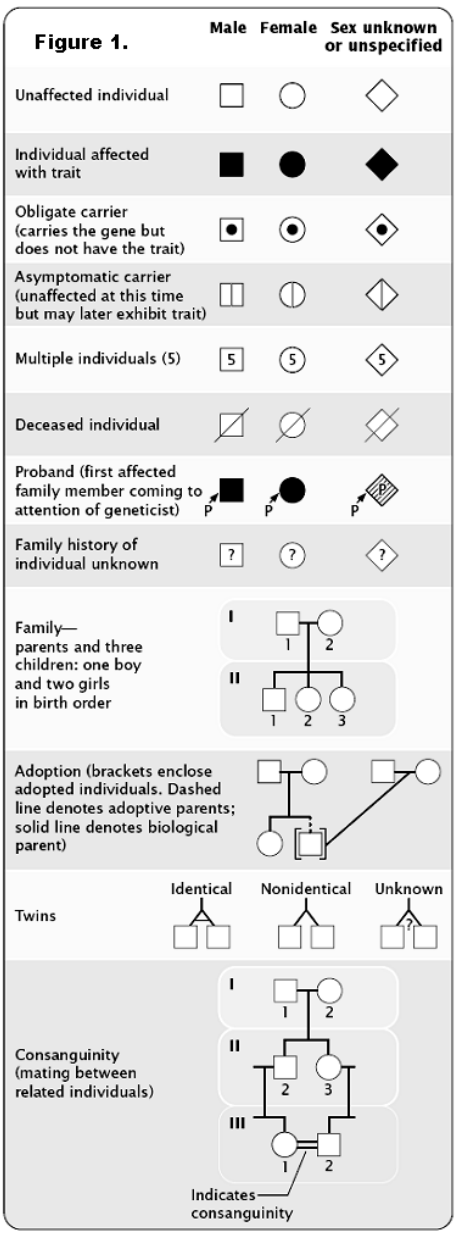
First, controlled matings are not possible. With other organisms, geneticists carry out specific crosses to test their hypotheses about inheritance. We have seen, for example, how the testcross provides a convenient way to determine if an individual with a dominant trait is homozygous or heterozygous. Unfortunately (for the geneticist at least), matings between humans are more frequently determined by romance, family expectations, and—occasionally—accident than they are by the requirements of the geneticist. Another obstacle is that humans have a long generation time. Human reproductive age is not normally reached until 10 to 14 years after birth, and most humans do not reproduce until they are 18 years of age or older; thus, generation time in humans is usually about 20 years. This long generation time means that, even if geneticists could control human crosses, they would have to wait on average 40 years just to observe the F2 progeny. In contrast, generation time in Drosophila is 2 weeks; in bacteria, it's a mere 20 minutes.

Finally, human family size is generally small. Observation of even the simple genetic ratios that we learned would require a substantial number of progeny in each family. When parents produce only 2 children, it's impossible to detect a 3:1 ratio. Even an extremely large family with 10 to 15 children would not permit the recognition of a dihybrid 9:3:3:1 ratio.

Although these special constraints make genetic studies of humans more complex, understanding human heredity is tremendously important. So geneticists have been forced to develop techniques that are uniquely suited to human biology and culture.

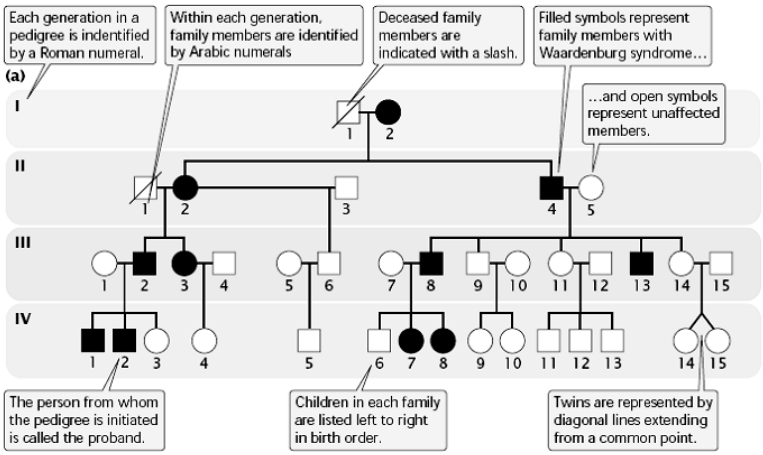
Analyzing Pedigrees

An important technique used by geneticists to study human inheritance is the pedigree. A pedigree is a pictorial representation of a family history, essentially a family tree that outlines the inheritance of one or more characteristics. The symbols commonly used in pedigrees are summarized in FIGURE 1. The pedigree shown in FIGURE 2a illustrates a family with Waardenburg syndrome, an autosomal dominant type of deafness that may be accompanied by fair skin, a white forelock, and visual problems (FIGURE 2b). Males in a pedigree are represented by squares, females by circles. A horizontal line drawn between two symbols representing a man and a woman indicates a mating; children are connected to their parents by vertical lines extending below the parents. Persons who exhibit the trait of interest are represented by filled circles and squares; in the pedigree of Figure 2a, the filled symbols represent members of the family who have Waardenburg syndrome. Unaffected persons are represented by open circles and squares.



Let's look closely at Figure 2 and consider some additional features of a pedigree. Each generation in a pedigree is identified by a Roman numeral; within each generation, family members are assigned Arabic numerals, and children in each family are listed in birth order from left to right.

Person II-4, a man with Waardenburg syndrome, mated with II-5, an unaffected woman, and they produced five children. The oldest of their children is III-8, a male with Waardenburg syndrome, and the youngest is III-14, an unaffected female. Deceased family members are indicated by a slash through the circle or square, as shown for I-1 and



2 Waardenburg syndrome is an autosomal dominant disease characterized by deafness, fair skin, visual problems, and a white forelock.

II-1 in Figure 2a. Twins are represented by diagonal lines extending from a common point (IV-14 and IV-15; non identical twins).

When a particular characteristic or disease is observed in a person, a geneticist studies the family of this affected person and draws a pedigree. The person from whom the pedigree is initiated is called the proband and is usually designated by an arrow (IV-I in Figure 2a). The limited number of offspring in most human families means that it is usually impossible to discern clear Mendelian ratios in a single pedigree. Pedigree analysis requires a certain amount of genetic sleuthing, based on recognizing patterns associated with different modes of inheritance. For example, autosomal dominant traits should appear with equal frequency in both sexes and should not skip generations, provided that the trait is fully penetrant and not sex influenced.

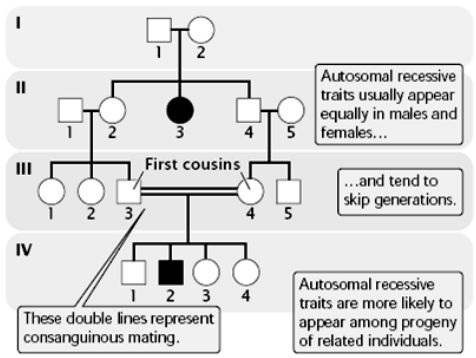
Certain patterns may exclude the possibility of a particular mode of inheritance. For instance, a son inherits his X chromosome from his mother. If we observe that a trait is passed from father to son, we can exclude the possibility of X-linked inheritance. In the following sections, the traits discussed are assumed to be fully penetrant and rare.

Autosomal Recessive Traits

Autosomal recessive traits normally appear with equal frequency in both sexes (unless penetrance differs in males and females), and appear only when a person inherits two alleles for the trait, one from each parent. If the trait is uncommon, most parents carrying the allele are heterozygous and unaffected; consequently, the trait appears to skip generations (FIGURE 3). Frequently, a recessive allele may be passed for a number of generations without the trait appearing in a pedigree. Whenever both parents are heterozygous, approximately ¼ of the offspring are expected to express the trait, but this ratio will not be obvious unless the family is large. In the rare event that both parents are affected by an autosomal recessive trait, all the offspring will be affected.

When a recessive trait is rare, persons from outside the family are usually homozygous for the normal allele. Thus, when an affected person mates with someone outside the family (aa X AA), usually none of the children will display the trait, although all will be carriers (i.e., heterozygous). A recessive trait is more likely to appear in a pedigree when two people within the same family mate, because there is a greater chance of both parents carrying the same recessive allele. Mating between closely related people is called consanguinity. In the pedigree shown in Figure 3, persons III-3 and III-4 are first cousins, and both are heterozygous for the recessive allele; when they mate, of their children ¼ are expected to have the recessive trait.

A number of human metabolic diseases are inherited as autosomal recessive traits. One of them is Tay-Sachs disease. Children with Tay-Sachs disease appear healthy at birth but become listless and weak at about 6 months of age. Gradually, their physical and neurological conditions worsen, leading to blindness, deafness, and eventually death at 2 to 3 years of age. The disease results from the accumulation of a lipid called G_{M2} ganglioside in the brain. A normal component of brain cells, G_{M2} ganglioside is usually broken down by an enzyme called hexosaminidase A, but children with Tay-Sachs disease lack this enzyme. Excessive G_{M2} ganglioside accumulates in the brain, causing swelling and, ultimately, neurological symptoms. Heterozygotes have only one normal copy of the hexosaminidase A allele and produce only about half the normal amount of the enzyme, but this amount is enough to ensure that G_{M2} ganglioside is broken down normally, and heterozygotes are usually healthy.

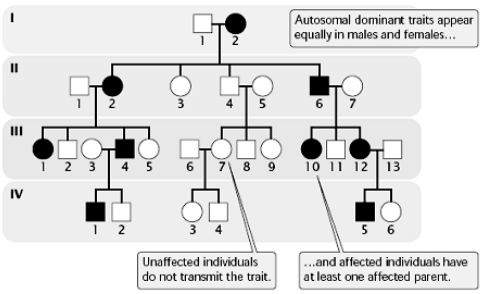


3 Autosomal recessive traits normally appear with equal frequency in both sexes and seem to skip generations.

Autosomal Dominant Traits

Autosomal dominant traits appear in both sexes with equal frequency, and both sexes are capable of transmitting these traits to their offspring. Every person with a dominant trait must inherit the allele from at least one parent; autosomal dominant traits therefore do not skip generations (FIGURE 4). Exceptions to this rule arise when people acquire the trait as a result of a new mutation or when the trait has reduced penetrance.

If an autosomal dominant allele is rare, most people displaying the trait are heterozygous. When one parent is affected and heterozygous and the other parent is unaffected, approximately of the offspring will be affected. If both parents have the trait and are heterozygous, approximately of the children will be affected. Provided the trait is fully penetrant, unaffected people do not transmit the trait to their descendants. In Figure 6.5, we see that none of the descendants of II-4 (who is unaffected) have the trait.



4 Autosomal dominant traits normally appear with equal frequency in both sexes and do not skip generations.

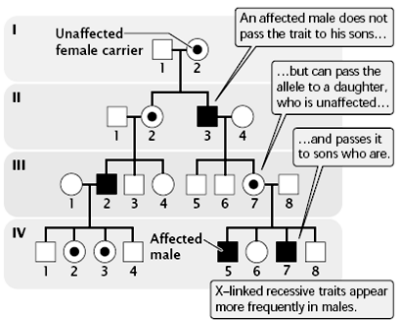
One trait usually considered to be autosomal dominant is familial hypercholesterolemia, an inherited disease in which blood cholesterol is greatly elevated owing to a defect in cholesterol transport. Cholesterol is an essential component of cell membranes and is used in the synthesis of bile salts and several hormones. Most of our cholesterol is obtained through foods, primarily those high in saturated fats. Because cholesterol is a lipid (a nonpolar, or uncharged, compound), it is not readily soluble in the blood (a polar, or charged, solution). Cholesterol must therefore be transported throughout the body in small soluble particles called lipoproteins; a lipoprotein consists of a core of lipid surrounded by a shell of charged phospholipids and proteins that dissolve easily in blood. One of the principle lipoproteins in the transport of cholesterol is low-density lipoprotein (LDL).When an LDL molecule reaches a cell, it attaches to an LDL receptor, which then moves the LDL through the cell membrane into the cytoplasm, where it is broken down and its cholesterol is released for use by the cell.

Familial hypercholesterolemia is due to a defect in the gene (located on human chromosome 19) that normally codes for the LDL receptor. The disease is usually considered an autosomal dominant disorder because heterozygotes are deficient in LDL receptors. In these people, too little cholesterol is removed from the blood, leading to elevated blood levels of cholesterol and increased risk of coronary artery disease. Persons heterozygous for familial hypercholesterolemia have blood LDL levels that are twice normal and usually have heart attacks by the age of 35. About 1 in 500 people is heterozygous for familial hypercholesterolemia and is predisposed to early coronary artery disease.

Very rarely, a person inherits two defective LDL receptor alleles. Such persons don't make any functional LDL receptors; their blood cholesterol levels are more than six times normal and they may suffer a heart attack as early as age 2 and almost inevitably by age 20. Because homozygotes are more severely affected than heterozygotes, familial hypercholesterolemia is said to be incompletely dominant. However, homozygotes are rarely seen (occurring with a frequency of only about 1 in 1 million people), and the common heterozygous form of the disease appears as a simple dominant trait in most pedigrees.

X-Linked Recessive Traits

X-linked recessive traits have a distinctive pattern of inheritance (FIGURE 5). First, these traits appear more frequently in males, because males need inherit only a single copy of the allele to display the trait, whereas females must inherit two copies of the allele, one from each parent, to be affected. Second, because a male inherits his X chromosome from his mother, affected males are usually born to unaffected mothers who carry an allele for the trait. Because the trait is passed from unaffected female to affected male to unaffected female, it tends to skip generations (see Figure 5). When a woman is heterozygous, approximately ½ of her sons will be affected and ½ of her daughters will be unaffected carriers. For example, we know that females I-2, II-2, and III-7 in Figure 6 are all carriers because they transmit the trait to approximately half of their sons.



5 X-linked recessive traits appear more often in males and are not passed from father to son.

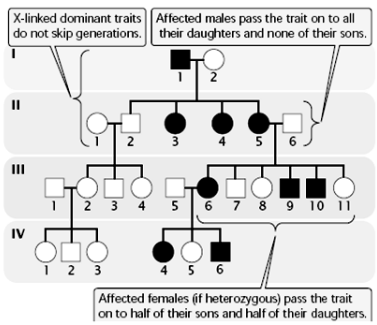
A third important characteristic of X-linked recessive traits is that they are not passed from father to son, because a son inherits his father's Y chromosome, not his X. In Figure 6, there is no case of a father and son who are both affected. All daughters of an affected man, however, will be carriers (if their mother is homozygous for the normal allele). When a woman displays an X-linked trait, she must be homozygous for the trait, and all of her sons will also display the trait.

An example of an X-linked recessive trait in humans is hemophilia A, also called classical hemophilia. This disease results from the absence of a protein necessary for blood to clot. The complex process of blood clotting consists of a cascade of reactions that includes more than 13 different factors. For this reason, there are several types of clotting disorders, each due to a glitch in a different step of the clotting pathway. Hemophilia A results from abnormal or missing factor VIII, one of the proteins in the clotting cascade. The gene for factor VIII is located on the tip of the long arm of the X chromosome; so hemophilia A is an X-linked recessive disorder. People with hemophilia A bleed excessively; even small cuts and bruises can be life threatening. Spontaneous bleeding occurs in joints such as elbows, knees, and ankles, which produces pain, swelling, and erosion of the bone. Fortunately, bleeding in people with

hemophilia A can be now controlled by administering concentrated doses of factor VIII.

X-Linked Dominant Traits

X-linked dominant traits appear in males and females, although they often affect more females than males. As with X-linked recessive traits, a male inherits an X-linked dominant trait only from his mother—the trait is not passed from father to son. A female, on the other hand, inherits an X chromosome from both her mother and father; so females can receive an X-linked trait from either parent. Each child with an X-linked dominant trait must have an affected parent (unless the child possesses a new mutation or the trait has reduced penetrance). X-linked dominant traits do not skip generations (FIGURE 6); affected men pass the trait on to all their daughters and none of their sons, as is seen in the children of I-1. In contrast, affected women (if heterozygous) ass the trait on to ½ of their sons and ½ of their daughters, as seen in the children of II-5 in the pedigree.

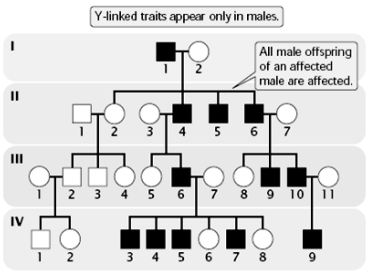


6. **X-linked dominant traits affect both males and females.** An affected male must have an affected mother.

An example of an X-linked dominant trait in humans is hypophosphatemia, also called familial vitamin D-resistant rickets. People with this trait have features that superficially resemble those produced by rickets: bone deformities, stiff spines and joints, bowed legs, and mild growth deficiencies. This disorder, however, is resistant to treatment with vitamin D, which normally cures rickets. X-linked hypophosphatemia results from the defective transport of phosphate, especially in cells of the kidneys. People with this disorder excrete large amounts of phosphate in their urine, resulting in low levels of phosphate in the blood and reduced deposition of minerals in the bone. As is common with X-linked dominant traits, males with hypophosphatemia are often more severely affected than females.

Y-Linked Traits

Y-linked traits exhibit a specific, easily recognized pattern of inheritance. Only males are affected, and the trait is passed from father to son. If a man is affected, all his male offspring should also be affected, as is the case for I-1, II-4, II-6, III-6, and III-10 of the pedigree in FIGURE 7. Y-linked traits do not skip generations. As discussed earlier comparatively few genes reside on the human Y chromosome, and so few human traits are Y linked.



7 **Y-linked traits appear only in males and are passed from a father to all his sons.**

The major characteristics of autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked traits are summarized in Table 1.

Table 1 Pedigree characteristics of autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant, and Y-linked traits

Autosomal recessive trait
1. Appears in both sexes with equal frequency.
2. Trait tends to skip generations.
3. Affected offspring are usually born to unaffected parents.
4. When both parents are heterozygous, approximately 1/4 of the offspring will be affected.
5. Appears more frequently among the children of consanguine marriages.

Autosomal dominant trait
1. Appears in both sexes with equal frequency.
2. Both sexes transmit the trait to their offspring.
3. Does not skip generations.
4. Affected offspring must have an affected parent, unless they possess a new mutation.
5. When one parent is affected (heterozygous) and the other parent is unaffected, approximately 1/2 of the offspring will be affected.
6. Unaffected parents do not transmit the trait.

X-linked recessive trait
1. More males than females are affected.
2. Affected sons are usually born to unaffected mothers; thus, the trait skips generations.
3. A carrier (heterozygous) mother produces approximately 1/2 affected sons.
4. Is never passed from father to son.
5. All daughters of affected fathers are carriers.

X-linked dominant trait
1. Both males and females are affected; often more females than males are affected.
2. Does not skip generations. Affected sons must have an affected mother; affected daughters must have either an affected mother or an affected father.
3. Affected fathers will pass the trait on to all their daughters.
4. Affected mothers (if heterozygous) will pass the trait on to 1/2 of their sons and 1/2 of their daughters.

Y-linked trait
1. Only males are affected.
2. Is passed from father to all sons.
3. Does not skip generations.

Twin Studies

Another method that geneticists use to analyze the genetics of human characteristics is twin studies. Twins come in two types: dizygotic (nonidentical) twins arise when two separate eggs are fertilized by two different sperm, producing genetically distinct zygotes; monozygotic (identical) twins result when a single egg, fertilized by a single sperm, splits early in development into two separate embryos. Because monozygotic twins arise from a single egg and sperm (a single, "mono," zygote), except for rare somatic mutations, they're genetically identical, having 100% of their genes in common. Dizygotic twins, on the other hand, have on average only 50% of their genes in common (the same percentage that any pair of siblings has in common). Like other siblings, dizygotic twins may be of the same or different sexes. The only difference between dizygotic twins and other siblings is that dizygotic twins are the same age and shared a common uterine environment.

The frequency with which dizygotic twins are born varies among populations. Among North American Caucasians, about 7 dizygotic twin pairs are born per 1000 births but, among Japanese, the rate is only about 3 pairs per 1000 births; among Nigerians, about 40 dizygotic twin pairs are born per 1000 births. The rate of dizygotic twinning also varies with maternal age, and dizygotic twinning tends to run in families. In contrast, monozygotic twinning is relatively constant. The frequency of monozygotic twinning in most ethnic groups is about 4 twin pairs per 1000 births, and there is relatively little tendency for monozygotic twins to run in families.

Concordance: Comparisons of dizygotic and monozygotic twins can be used to estimate the importance of genetic and environmental factors in producing differences in a characteristic. This is often done by calculating the concordance for a trait. If both members of a twin pair have a trait, the twins are said to be concordant; if only one member of the pair has the trait, the twins are said to be discordant. Concordance is the percentage of twin pairs that are concordant for a trait. Because identical twins have 100% of their genes in common and dizygotic twins have on average only 50% in common, genetically influenced traits should exhibit higher concordance in monozygotic twins. For instance, when one member of a monozygotic twin pair has asthma, the other twin of the pair has asthma about 48% of the time, so the monozygotic concordance for asthma is 48%. However, when a dizygotic twin has asthma, the other twin has asthma only 19% of the time (19% dizygotic concordance). The higher concordance in the monozygotic twins suggests that genes influence asthma, a finding supported by other family studies of this disease.

The hallmark of a genetic influence on a particular characteristic is higher concordance in monozygotic twins compared with concordance in dizygotic twins. High concordance in monozygotic twins by itself does not signal a genetic influence. Twins normally share the same environment— they are raised in the same home, have the same friends, attend the same school—so high concordance may be due to common genes or to common environment. If the high concordance is due to environmental factors, then dizygotic twins, who also share the same environment, should have just as high a concordance as that of monozygotic twins. When genes influence the characteristic, however, monozygotic twin pairs should exhibit higher concordance than dizygotic twin pairs, because monozygotic twins have a greater percentage of genes in common. It is important to note that any discordance among monozygotic twins must be due to environmental factors, because monozygotic twins are genetically identical. The use of twins in genetic research rests on the important assumption that, when there is greater concordance in monozygotic twins than in dizygotic twins, it is because monozygotic twins are more similar in their genes and not because they have experienced a more similar environment.

The degree of environmental similarity between monozygotic twins and dizygotic twins is assumed to be the same. This assumption may not always be correct, particularly for human behaviors. Because they look alike, identical twins may be treated more similarly by parents, teachers, and peers than are nonidentical twins. Evidence of this similar treatment is seen in the past tendency of parents to dress identical twins alike. In spite of this potential complication, twin studies have played a pivotal role in the study of human genetics.

GENETIC AND METABOLIC DISORDERS

1. Severe combined immunodeficiency

Severe combined immunodeficiency (SCID) represents a group of rare, sometimes fatal, congenital disorders characterized by little or no immune response. The defining feature of SCID, commonly known as "bubble boy" disease, is a defect in the specialized white blood cells (B- and T-lymphocytes) that defend us from infection by viruses, bacteria and fungi. Without a functional immune system, SCID patients are susceptible to recurrent infections such as pneumonia, meningitis and chicken pox, and can die before the first year of life. Though invasive, new treatments such as bone marrow and stem-cell transplantation save as many as 80% of SCID patients. All forms of SCID are inherited, with as many as half of SCID cases linked to the X chromosome, passed on by the mother. X-linked SCID results from a mutation in the interleukin 2 receptor gamma (IL2RG) gene. In another form of SCID, there is a lack of the enzyme adenosine deaminase (ADA), coded for by a gene on chromosome 20. This means that the substrates for this enzyme accumulate in cells. Immature lymphoid cells of the immune system are particularly sensitive to the toxic effects of these unused substrates, so fail to reach maturity. As a result, the immune system of the afflicted individual is severely compromised or completely lacking.

2. Hereditary hemochromatosis

Hereditary hemochromatosis is an inherited disorder that increases the amount of iron that the body absorbs from the

gut. Symptoms are caused by this excess iron being deposited in multiple organs of the body. Most commonly, excess iron in the liver causes cirrhosis, which may develop into liver cancer. Iron deposits in the pancreas can result in diabetes. Similarly, excess iron stores can cause cardiomyopathy, pigmentation of the skin, and arthritis.

Many mutations in the body's iron transport system can cause hemochromatosis; however, most cases are caused by mutations in the HFE gene. This is located on chromosome 6, and one mutation leads to the substitution of the 282nd amino acid, Cysteine becomes tyrosine, therefore the mutation is called C282Y. Hemochromatosis is one of the most common autosomal recessive disorders among Caucasians in the United States; however, only a small proportion of these people suffer any symptoms. This may be attributable to both environmental (diet and blood loss) and genetic factors.

3. Alzheimer disease (AD)

Alzheimer disease (AD) is the fourth leading cause of death in adults. The incidence of the disease rises steeply with age. Alzheimer disease is twice as common in women than in men. Some of the most frequently observed symptoms of the disease include a progressive inability to remember facts and events and, later, to recognize friends and family.

AD tends to run in families; currently, mutations in four genes, situated on chromosomes 1, 14, 19, and 21, are believed to play a role in the disease. The best-characterized of these are PS1 (or AD3) on chromosome 14 and PS2 (or AD4) on chromosome 1. The formation of lesions made of fragmented brain cells surrounded by β-amyloid-family proteins are characteristic of the disease.

4. Huntington disease

Huntington disease (HD) is an inherited, degenerative neurological disease that leads to dementia. The HD gene, whose mutation results in Huntington disease, was mapped to chromosome 4. The mutation is a characteristic expansion of a nucleotide triplet repeat in the DNA that codes for the protein huntingtin. As the number of repeated triplets - CAG (cytosine, adenine, guanine) - increases, the age of onset in the patient decreases. Furthermore, because the unstable trinucleotide repeat can lengthen when passed from parent to child, the age of onset can decrease from one generation to the next. Since people who have those repeats always suffer from Huntington disease, it suggests that the mutation causes a gain-of-function, in which the mRNA or protein takes on a new property or is expressed inappropriately.

5. Parkinson disease

Parkinson disease, first described by James Parkinson in 1817, is a neurodegenerative disease that manifests as a tremor, muscular stiffness and difficulty with balance and walking. A classic pathological feature of the disease is the presence of an inclusion body, called the Lewy body, in many regions of the brain.

Parkinson disease was mapped to chromosome 4. Mutations in this gene have now been linked to several Parkinson disease families. The product of this gene, a protein called alpha-synuclein, is a familiar culprit: a fragment of it is a known constituent of Alzheimer disease plaques.

6. Thalassemia

Thalassemia is an inherited disease of faulty synthesis of hemoglobin. It consists of a group of disorders that may range from a barely detectable abnormality of blood, to severe or fatal anemia. Adult hemoglobin is composed of two alpha (a) and two beta (b) polypeptide chains. There are two copies of the hemoglobin alpha gene (HBA1 and HBA2), which each encode an α-chain, and both genes are located on chromosome 16. The hemoglobin beta gene (HBB) encodes the β-chain and is located on chromosome 11.

In α -thalassemia, there is deficient synthesis of α -chains. The resulting excess of β-chains bind oxygen poorly, leading to a low concentration of oxygen in tissues (hypoxemia). Similarly, in β-thalassemia there is a lack of β -chains. However, the excess α-chains can form insoluble aggregates inside red blood cells. These aggregates cause the death of red blood cells and their precursors, causing a very severe anemia. The spleen becomes enlarged as it removes damaged red blood cells from the circulation.

Currently, severe thalassemia is treated by blood transfusions, and a minority of patients are cured by bone marrow transplantation. Mouse models are proving to be useful in assessing the potential of gene therapy

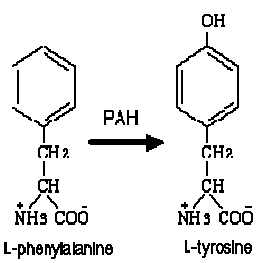
7. Cystic fibrosis (CF)

Cystic fibrosis (CF) is the most common fatal genetic disease. It causes the body to produce a thick, sticky mucus that clogs the lungs, leading to infection, and blocks the pancreas, stopping digestive enzymes from reaching the intestines where they are required to digest food.

CF is caused by a defective gene, which codes for a sodium and chloride (salt) transporter found on the surface of the epithelial cells that line the lungs and other organs. Several hundred mutations have been found in this gene, all of which result in defective transport of sodium and chloride by epithelial cells. The severity of the disease symptoms of CF is directly related to the characteristic effects of the particular mutation(s) that have been inherited by the sufferer.

8. Phenylketonuria

Phenylketonuria (PKU) is an inherited error of metabolism caused by a deficiency in the enzyme phenylalanine hydroxylase. Loss of this enzyme results in mental retardation, organ damage, unusual posture and can, in cases of maternal PKU, severely compromise pregnancy.



Classical PKU is an autosomal recessive disorder, caused by mutations in both alleles of the gene for phenylalanine hydroxylase (PAH), found on chromosome 12. In the body, phenylalanine hydroxylase converts the amino acid phenylalanine to tyrosine, another amino acid. Mutations in both copies of the gene for PAH means that the enzyme is inactive or is less efficient, and the concentration of phenylalanine in the body can build up to toxic levels and converts into phenyl pyruvic acid which deposit in brain and causes mental retardation.

9. Tay-Sachs disease

Tay-Sachs disease, a heritable metabolic disorder commonly associated with Ashkenazi Jews, also found in other populations throughout the world. The severity of expression and the age at onset of Tay-Sachs varies from infantile and juvenile forms that exhibit paralysis, dementia, blindness and early death to a chronic adult form that exhibits neuron dysfunction and psychosis.

Tay-Sachs is an autosomal recessive disease caused by mutations in both alleles of a gene (HEXA) on chromosome 15. HEXA codes for the alpha subunit of the enzyme β-hexosaminidase A. This enzyme is found in lysosomes, organelles that break down large molecules for recycling by the cell. Normally, β-hexosaminidase A helps to degrade a lipid called GM2 ganglioside, but in Tay-Sachs individuals, the enzyme is absent or present only in very reduced amounts, allowing excessive accumulation of the GM2 ganglioside in neurons. The progressive neurodegeneration seen in the varied forms of Tay-Sachs depends upon the speed and degree of GM2 ganglioside accumulation, which in turn is dependent upon the level of functional β-hexosaminidase A present in the body.

10. Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is one of a group of muscular dystrophies characterized by the enlargement of muscles. All are X-linked and affect mainly males—an estimated 1 in 3500 boys worldwide.The gene for DMD, found on the X chromosome, encodes a large protein—dystrophin. Dystrophin is required inside muscle cells for structural support; Without it, the cell membrane becomes permeable, so that extracellular components enter the cell, increasing the internal pressure until the muscle cell "explodes" and dies. The subsequent immune response can add to the damage.

11. Lesch-Nyhan syndrome

Lesch-Nyhan syndrome (LNS) is a rare inherited disease that disrupts the metabolism of the raw material of genes. These raw materials are called purines, and they are an essential part of DNA and RNA. The body can either make purines (de novo synthesis) or recycle them (the resalvage pathway). Many enzymes are involved in these pathways. When one of these enzymes is missing, a wide range of problems can occur.

In LNS, there is a mutation in the HPRT1 gene located on the X chromosome. The product of the normal gene is the enzyme hypoxanthine-guanine phosphoribosyltransferase, which speeds up the recycling of purines from broken down DNA and RNA. Many different types of mutations affect this gene, and the result is a very low level of the enzyme.

The mutation is inherited in an X-linked fashion. Females who inherit one copy of the mutation are not affected because they have two copies of the X chromosome (XX). Males are severely affected because they only have one X chromosome (XY), and therefore their only copy of the HPRT1 gene is mutated.

12. Hemophilia A:

Hemophilia A is a hereditary blood disorder, primarily affecting males, characterized by a deficiency of the blood clotting protein known as Factor VIII that results in abnormal bleeding. Mutation of the HEMA gene on the X chromosome causes Hemophilia A in reccesive homozygous condition. Normally, females have two X chromosomes, whereas males have one X and one Y chromosome. Since males have only a single copy of any gene located on the X chromosome, they cannot offset damage to that gene with an additional copy as can females. Consequently, X-linked disorders such as Hemophilia A are far more common in males. The HEMA gene codes for Factor VIII, which is synthesized mainly in the liver, and is one of many factors involved in blood coagulation; its loss alone is enough to cause Hemophilia A even if all the other coagulation factors are still present.

13. SRY: Sex determination

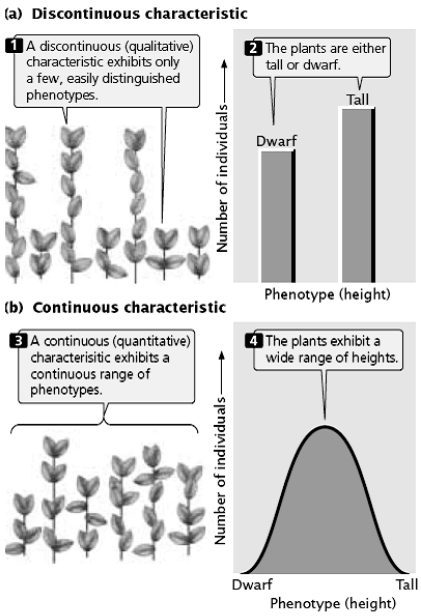
Usually, a woman has two X chromosomes (XX) and a man one X and one Y (XY). However, both male and female characteristics can sometimes be found in one individual, and it is possible to have XY women and XX men. Analysis of such individuals has revealed some of the molecules involved in sex determination, including one called SRY, which is important for testis formation.

SRY (which stands for sex-determining region Y gene) is found on the Y chromosome. In the cell, it binds to other DNA and in doing so distorts it dramatically out of shape. This alters the properties of the DNA and likely alters the expression of a number of genes, leading to testis formation. Most XX men who lack a Y chromosome do still have a copy of the SRY region on one of their X chromosomes. This copy accounts for their maleness. However, because the remainder of the Y chromosome is missing they frequently do not develop secondary sexual characteristics in the usual way.

H. Quantitative genetics: Polygenic inheritance, heritability and its measurements, QTL mapping.

Quantitative Characteristics

Qualitative, or discontinuous, characteristics possess only a few distinct phenotypes (FIGURE 1a); these characteristics are the types studied by Mendel and have been the focus of our attention thus far. However, many characteristics vary continuously along a scale of measurement with many overlapping phenotypes (FIGURE 1b). They are referred to as continuous characteristics; they are also called quantitative characteristics because any individual's phenotype must be described with a quantitative measurement. Quantitative characteristics might include height, weight, and blood pressure in humans, growth rate in mice, seed weight in plants, and milk production in cattle. Quantitative characteristics arise from two phenomena. First, many are polygenic—they are influenced by genes at many loci. If many loci take part, many genotypes are possible, each producing a slightly different phenotype. Second, quantitative characteristics often arise when environmental factors affect the phenotype, because environmental differences result in a single genotype producing a range of phenotypes. Most continuously varying characteristics are both polygenic and influenced by environmental factors, and these characteristics are said to be multifactorial.



1 **Discontinuous and continuous characteristics differ in the number of phenotypes exhibited.**

The Relation Between Genotype and Phenotype

For many discontinuous characteristics, there is a relatively straightforward relation between genotype and phenotype. Each genotype produces a single phenotype, and most phenotypes are encoded by a single genotype. Dominance and epistasis may allow two or three genotypes to produce the same phenotype, but the relation remains relatively simple. This simple relation between genotype and phenotype allowed Mendel to decipher the basic rules of inheritance from his crosses with pea plants; it also permits us both to predict the outcome of genetic crosses and to assign genotypes to individuals.

For quantitative characteristics, the relation between genotype and phenotype is often more complex. If the characteristic is polygenic, many different genotypes are possible, several of which may produce the same phenotype. For instance, consider a plant whose height is determined by three loci (A, B, and C), each of which has two alleles. Assume that one allele at each locus (A^+, B^+, and C^+) encodes a plant hormone that causes the plant to grow 1 cm above its baseline height of 10 cm. The second allele at each locus (A^-, B^-, and C^-) encodes no plant hormone and does not contribute to additional height. Considering only the two alleles at a single locus, 3 genotypes are possible (A^+A^+, A^+A^-, and A^-A^-). If all three loci are taken into account, there are a total of $3^3 = 27$ possible multilocus genotypes ($A^+A^+B^+B^+C^+C^+$, $A^+A^-B^+B^+C^+C^+$, etc.). Although there are 27 genotypes, they produce only seven phenotypes (10 cm, 11 cm, 12 cm, 13 cm, 14 cm, 15 cm, and 16 cm in height). Some of the genotypes produce the same phenotype (Table 1); for example, genotypes $A^+A^-B^-B^-C^-C^-$, $A^-A^-B^+B^-C^-C^-$ and $A^-A^-B^-B^-C^+C^-$ all have one gene that encodes plant hormone.

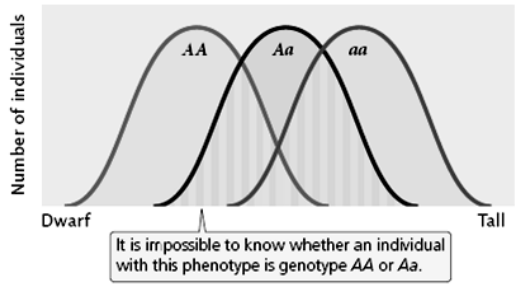
Table 1 Hypothetical example of plant height determined by pairs of alleles at each of three loci

Genotype	Doses of Plant Hormone	Height (cm)
$A^-A^-B^-B^-C^-C^-$	0	10
$A^+A^-B^-B^-C^-C^-$ $A^-A^-B^+B^-C^-C^-$ $A^-A^-B^-B^-C^-C^+$	1	11
$A^+A^+B^-B^-C^-C^-$ $A^-A^-B^+B^+C^-C^-$ $A^-A^-B^-B^-C^+C^+$ $A^+A^-B^+B^-C^-C^-$ $A^+A^-B^-B^-C^+C^-$ $A^-A^-B^+B^-C^+C^-$	2	12
$A^+A^+B^+B^-C^-C^-$ $A^+A^+B^-B^-C^+C^-$ $A^+A^-B^+B^+C^-C^-$ $A^-A^-B^+B^+C^+C^-$ $A^+A^-B^-B^-C^+C^+$ $A^-A^-B^+B^-C^+C^+$ $A^+A^-B^+B^-C^+C^-$	3	13
$A^+A^+B^+B^+C^-C^-$ $A^+A^+B^+B^-C^+C^-$ $A^+A^-B^+B^+C^+C^-$ $A^-A^-B^+B^+C^+C^+$ $A^+A^+B^-B^-C^+C^+$ $A^-A^-B^+B^-C^+C^+$	4	14
$A^+A^+B^+B^+C^+C^-$ $A^+A^-B^+B^+C^+C^+$ $A^+A^+B^+B^-C^+C^+$	5	15
$A^+A^+B^+B^+C^+C^+$	6	16

Note: Each + allele contributes 1 cm in height above a baseline of 10 cm.

These genotypes produce one dose of the hormone and a plant that is 11 cm tall. Even in this simple example of only three loci, the relation between genotype and phenotype is

quite complex. The more loci encoding a characteristic, the greater the complexity. The influence of environment on a characteristic also can complicate the relation between genotype and phenotype. Because of environmental effects, the same genotype may produce a range of potential phenotypes (the norm of reaction). The phenotypic ranges of different genotypes may overlap, making it difficult to know whether individuals differ in phenotype because of genetic or environmental differences (FIGURE 2).



.2 **For a quantitative characteristic, each genotype may produce a range of possible phenotypes.** In this hypothetical example, the phenotypes produced by genotypes *AA*, *Aa*, and *aa* overlap.

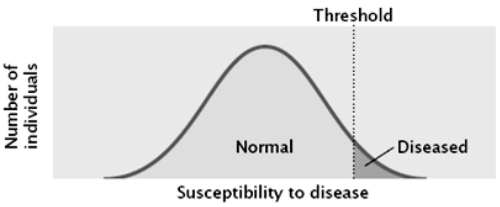
In summary, the simple relation between genotype and phenotype that exists for many qualitative (discontinuous) characteristics is absent in quantitative characteristics, and it is impossible to assign a genotype to an individual on the basis of its phenotype alone. The methods used for analyzing qualitative characteristics (examining the phenotypic ratios of progeny from a genetic cross) will not work with quantitative characteristics. Our goal remains the same: we wish to make predictions about the phenotypes of offspring produced in a genetic cross. We may also want to know how much of the variation in a characteristic results from genetic differences and how much results from environmental differences. To answer these questions, we must turn to statistical methods that allow us to make predictions about the inheritance of phenotypes in the absence of information about the underlying genotypes.

Types of Quantitative Characteristics

Before we look more closely at polygenic characteristics and relevant statistical methods, we need to more clearly define what is meant by a quantitative characteristic. Thus far, we have considered only quantitative characteristics that vary continuously in a population. A continuous characteristic can theoretically assume any value between two extremes; the number of phenotypes is limited only by our ability to precisely measure the phenotype. Human height is a continuous characteristic because, within certain limits, people can theoretically have any height. Although the number of phenotypes possible with a continuous characteristic is infinite, we often group similar phenotypes together for convenience; we may say that two people are both 5 feet 11 inches tall, but careful measurement may show that one is slightly taller than the other.

Some characteristics are not continuous but are nevertheless considered quantitative because they are determined by multiple genetic and environmental factors. Meristic characteristics, for instance, are measured in whole numbers. An example is litter size: a female mouse may have 4, 5, or 6 pups but not 4.13 pups. A meristic characteristic has a limited number of distinct phenotypes, but the underlying determination of the characteristic may still be quantitative. These characteristics must therefore be analyzed with the same techniques that we use to study continuous quantitative characteristics.

Another type of quantitative characteristic is a threshold characteristic, which is simply present or absent. Although threshold characteristics exhibit only two phenotypes, they are considered quantitative because they, too, are determined by multiple genetic and environmental factors. The expression of the characteristic depends on an underlying susceptibility (usually referred to as liability or risk) that varies continuously. When the susceptibility is larger than a threshold value, a specific trait is expressed (FIGURE 3). Diseases are often threshold characteristics because many factors, both genetic and environmental, contribute to disease susceptibility. If enough of the susceptibility factors are present, the disease develops; otherwise, it is absent. Although we focus on the genetics of continuous characteristics in this topic, the same principles apply to many meristic and threshold characteristics.



3 **Threshold characteristics display only two possible phenotypes—the trait is either present or absent—but they are quantitative because the underlying susceptibility to the characteristic varies continuously.** When the susceptibility exceeds a threshold value, the characteristic is expressed.

It is important to point out that just because a characteristic can be measured on a continuous scale does not mean that it exhibits quantitative variation. One of the characteristics studied by Mendel was height of the pea plant, which can be described by measuring the length of the plant's stem. However, Mendel's particular plants exhibited only two distinct phenotypes (some were tall and others short), and these differences were determined by alleles at a single locus. The differences that Mendel studied were therefore discontinuous in nature.

Polygenic Inheritance

The rediscovery of Mendel's work in 1900 provided a cohesive theory of inheritance, but the characteristics that Mendel studied were all discontinuous. Questions soon arose about the inheritance of continuously varying characteristics. These characteristics had already been the focus of a group of biologists and statisticians, led by Francis Galton, who were known as biometricians. They examined the inheritance of quantitative characteristics such as human height and intelligence by using statistical procedures. The results of these studies showed that quantitative

characteristics are inherited, although the mechanism of inheritance was as yet unknown. After Mendel's work was rediscovered, a bitter dispute broke out about whether Mendel's principles applied to quantitative characteristics. Some biometricians argued that the inheritance of quantitative characteristics could not be explained by Mendelian principles, whereas others felt that Mendel's principles acting on numerous genes (polygenes) could adequately account for the inheritance of quantitative characteristics.

This conflict began to be resolved by the work of Wilhelm Johannsen, who showed that continuous variation in the weight of beans was influenced by both genetic and environmental factors. George Udny Yule, a mathematician, proposed in 1906 that several genes acting together could produce continuous characteristics. This hypothesis was later confirmed by Herman Nilsson-Ehle, working on wheat and tobacco, and by Edward East, working on corn. The argument was finally laid to rest in 1918, when Ronald Fisher demonstrated that the inheritance of quantitative characteristics could indeed be explained by the cumulative effects of many genes, each following Mendel's rules.

Kernel Color in Wheat

To illustrate how multiple genes acting on a characteristic can produce a continuous range of phenotypes, let us examine one of the first demonstrations of polygenic inheritance. Nilsson-Ehle studied kernel color in wheat and found that the intensity of red pigmentation was determined by three unlinked loci, each of which had two alleles. Nilsson-Ehle obtained several homozygous varieties of wheat that differed in color. Like Mendel, he performed crosses between these homozygous varieties and studied the ratios of phenotypes in the progeny. In one experiment, he crossed a variety of wheat that possessed white kernels with a variety that possessed purple (very dark red) kernels and obtained the following results:

P: plants with white kernels × plants with purple kernels

↓

F_1: plants with red kernels

↓

F_2:
- 1/16 plants with purple kernels
- 4/16 plants with dark-red kernels
- 6/16 plants with red kernels
- 4/16 plants with light-red kernels
- 1/16 plants with white kernels

Nilsson-Ehle interpreted this phenotypic ratio as the result of segregation of alleles at two loci. (Although he found alleles at three loci that affected kernel color, the two varieties used in this cross differed only at two of the loci.)

He proposed that there were two alleles at each locus: one that produced red pigment and another that produced no pigment. We'll designate the alleles that encoded pigment A^+ and B^+ and the alleles that encoded no pigment A^- and B^-. Nilsson-Ehle recognized that the effects of the genes were additive. Each gene seemed to contribute equally to color; so the overall phenotype could be determined by adding the effects of all the genes, as shown in this table.

Genotype	Doses of pigment	Phenotype
$A^+A^+B^+B^+$	4	purple
$A^+A^+B^+B^-$ $A^+A^-B^+B^+$	3	dark red
$A^+A^+B^-B^-$ $A^-A^-B^+B^+$ $A^+A^-B^+B^-$	2	red
$A^+A^-B^-B^-$ $A^-A^-B^+B^-$	1	light red
$A^-A^-B^-B^-$	0	white

Notice that the purple and white phenotypes are each encoded by a single genotype, but other phenotypes may result from several different genotypes. From these results, we see that five phenotypes are possible when alleles at two loci influence the phenotype and the effects of the genes are additive. When alleles at more than two loci influence the phenotype, more phenotypes are possible, and this would make the color appear to vary continuously between white and purple. If environmental factors had influenced the characteristic, individuals of the same genotype would vary somewhat in color, making it even more difficult to distinguish between discrete phenotypic classes. Luckily, environment played little role in determining kernel color in Nilsson-Ehle's crosses, and only a few loci encoded color; so Nilsson-Ehle was able to distinguish among the different phenotypic classes. This ability allowed him to see the Mendelian nature of the characteristic.

Let's now see how Mendel's principles explain the ratio obtained by Nilsson-Ehle in his F2 progeny. Remember that Nilsson-Ehle crossed a homozygous purple variety ($A^+ A^+ B^+ B^+$) with the homozygous white variety ($A^- A^- B^- B^-$), producing F1 progeny that were heterozygous at both loci ($A^+ A^- B^+ B^-$). All the F1 plants possessed two pigment-producing alleles that allowed two doses of color to make red kernels. The types and proportions of progeny expected in the F2 can be found by applying Mendel's principles of segregation and independent assortment.

Let's first examine the effects of each locus separately. At the first locus, two heterozygous F1s are crossed ($A^+ A^- \times A^+ A^-$). As we learned, when two heterozygotes are crossed, we expect progeny in the proportions ¼ $A^+ A^+$, ½ $A^+ A^-$, and ¼ $A^- A^-$. At the second locus, two heterozygotes also are crossed, and again we expect progeny in the proportions ¼ $B^+ B^+$, ½ $B^+ B^-$, and ¼ $B^- B^-$.

To obtain the probability of combinations of genes at both loci, we must use the multiplication rule of probability, which is based on Mendel's principle of independent assortment. The expected proportion of F2 progeny with genotype $A^+ A^+ B^+ B^+$ is the product of the probability of obtaining genotype $A^+ A^+$ (1/4) and the probability of obtaining genotype B^+B^+ (1/4), or ¼ x ¼ =1/16(FIGURE 4). The probabilities of each of the phenotypes can then be obtained by adding the probabilities of all the genotypes that produce that phenotype. For example, the red phenotype is produced by three genotypes:

Genotype	Probability
$A^+A^+B^-B^-$	1/16
$A^-A^-B^+B^+$	1/16
$A^+A^-B^+B^-$	1/4

Thus, the overall probability of obtaining red kernels in the F2 progeny is 1/16 + 1/16 +1/4 =6/16 .

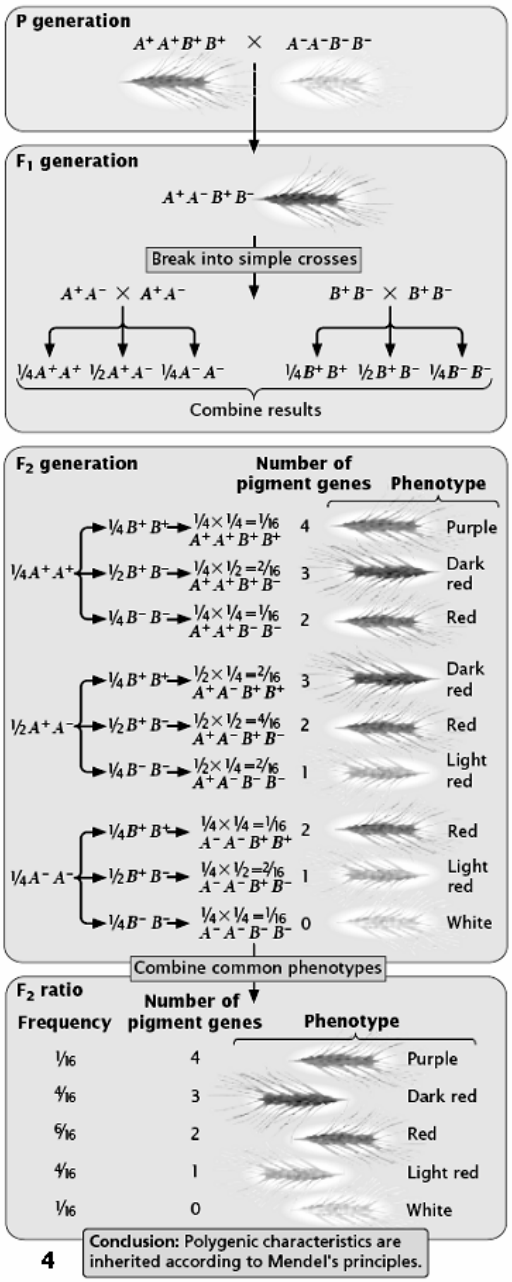
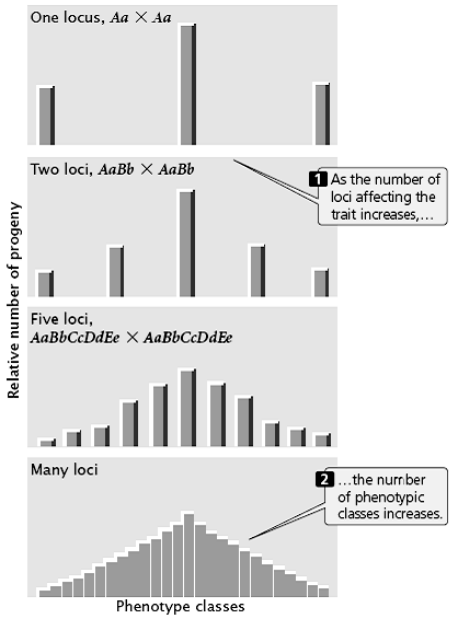


Figure shows that the phenotypic ratio expected in the F2 is 1/16 purple, 4/16 dark red, 6/16 red, 4/16 light red, and 1/16 white. This phenotypic ratio is precisely what Nilsson-Ehle observed in his F2 progeny, demonstrating that the inheritance of a continuously varying characteristic such as kernel color is indeed according to Mendel's basic principles. Nilsson-Ehle's crosses demonstrated that the difference between the inheritance of genes influencing quantitative characteristics and the inheritance of genes influencing discontinuous characteristics is in the number of loci that determine the characteristic. When multiple loci affect a character, more genotypes are possible; so the relation between the genotype and the phenotype is less obvious. As the number of loci affecting a character increases, the number of phenotypic classes in the F2 increases (FIGURE 5).



5 **The results of crossing individuals heterozygous for different numbers of loci affecting a characteristic.**

Several conditions of Nilsson-Ehle's crosses greatly simplified the polygenic inheritance of kernel color and made it possible for him to recognize the Mendelian nature of the characteristic. First, genes affecting color segregated at only two or three loci. If genes at many loci had been segregating, he would have had difficulty in distinguishing the phenotypic classes. Second, the genes affecting kernel color had strictly additive effects, making the relation between genotype and phenotype simple. Third, environment played almost no role in the phenotype; had environmental factors modified the phenotypes, distinguishing between the five phenotypic classes would have been difficult. Finally, the loci that Nilsson-Ehle studied were not linked; so the genes assorted independently. Nilsson-Ehle was fortunate—for many polygenic characteristics, these simplifying conditions are not present and Mendelian inheritance of these characteristics is not obvious.

Determining Gene Number for a Polygenic Characteristic

When two individuals homozygous for different alleles at a single locus are crossed (A^1A^1 x A^2A^2) and the resulting F1 are interbred (A^1A^2 x A^1A^2), one-fourth of the F2 should be homozygous like each of the original parents. If the original parents are homozygous for different alleles at two loci, as are those in Nilsson-Ehle's crosses, then ¼ x ¼ =1/16 of the F2 should resemble one of the original homozygous parents. Generally, $(1/4)^n$ will be the number of individuals in the F2 progeny that should resemble each of the original homozygous parents, where n equals the number of loci with a segregating pair of alleles that affects the

characteristic. This equation provides us with a possible means of determining the number of loci influencing a quantitative characteristic.

To illustrate the use of this equation, assume that we cross two different homozygous varieties of pea plants that differ in height by 16 cm, interbreed the F1, and find that approximately 1/256 of the F2 are similar to one of the original homozygous parental varieties. This outcome would suggest that 4 loci with segregating pairs of alleles ($1/256 = 1/4^4$) are responsible for the height difference between the two varieties. Because the two homozygous strains differ in height by 16 cm and there are 4 loci each with two alleles (8 alleles in all), each of the alleles contributes 16 cm/8 = 2 cm in height. This method for determining the number of loci affecting phenotypic differences requires the use of homozygous strains, which may be difficult to obtain in some organisms. It also assumes that all the genes influencing the characteristic have equal effects, that their effects are additive, and that the loci are unlinked. For many polygenic characteristics, these assumptions are not valid, so this method of determining the number of genes affecting a characteristic has limited application.

HERITABILITY

In addition to being polygenic, quantitative characteristics are frequently influenced by environmental factors. It is often useful to know how much of the variation in a quantitative characteristic is due to genetic differences and how much is due to environmental differences. That proportion of the total phenotypic variation that is due to genetic differences is known as the heritability.

Consider a dairy farmer who owns several hundred milk cows. The farmer notices that some cows consistently produce more milk than others. The nature of these differences is important to the profitability of his dairy operation. If the differences in milk production are largely genetic in origin, then the farmer may be able to boost milk production by selectively breeding the cows that produce the most milk. On the other hand, if the differences are largely environmental in origin, selective breeding will have little effect on milk production, and the farmer might better boost milk production by adjusting the environmental factors associated with higher milk production. To determine the extent of genetic and environmental influences on variation in a characteristic, phenotypic variation in the characteristic must be partitioned into components attributable to different factors.

Phenotypic Variance

To determine how much of phenotypic differences in a population is due to genetic and environmental factors, we must first have some quantitative measure of the phenotype under consideration. Consider a population of wild plants that differ in size. We could collect a representative sample of plants from the population, weigh each plant in the sample, and calculate the mean and variance of plant weight. This phenotypic variance is represented by VP.

Components of phenotypic variance

Phenotypic variance, which represents the phenotypic differences among individual members of a group, can be attributed to several factors. First, some of the differences in phenotype may be due to differences in genotypes among individual members of the population. These differences are termed the genetic variance and are represented by V_G. Second, some of the differences in phenotype may be due to environmental differences among the plants; these differences are termed the environmental variance, V_E. Environmental variance includes differences that can be attributed to specific environmental factors, such as the amount of light or water that the plant receives; it also includes random differences in development that cannot be attributed to any specific factor. Any variation in phenotype that is not inherited is, by definition, a part of the environmental variance.

Third, genetic–environmental interaction variance (V_{GE}) arises when the effect of a gene depends on the specific environment in which it is found. An example, in a dry environment, genotype AA produces a plant that averages 12 g in weight, and genotype aa produces a smaller plant that averages 10 g. In a wet environment, genotype aa produces the larger plant, averaging 24 g in weight, whereas genotype AA produces a plant that averages 20 g. In this example, there are clearly differences in the two environments: both genotypes produce heavier plants in the wet environment. There are also differences in the weights of the two genotypes, but the relative performances of the genotypes depend on whether the plants are grown in a wet or dry environment. In this case, the influences on phenotype cannot be neatly allocated into genetic and environmental components, because the expression of the genotype depends on the environment in which the plant grows. The phenotypic variance must therefore include a component that accounts for the way in which genetic and environmental factors interact.

In summary, the total phenotypic variance can be apportioned into three components:

$$V_P + V_G + V_E = V_{GE}$$

Components of genetic variance

Genetic variance can be further subdivided into components consisting of different types of genetic effects. First, additive genetic variance (V_A) comprises the additive effects of genes on the phenotype, which can be summed to determine the overall effect on the phenotype. For example, suppose that, in a plant, allele A^1 contributes 2 g in weight and allele A^2 contributes 4 g. If the alleles are strictly additive, then the genotypes would have the following weights:

$$A^1A^1 = 2 + 2 = 4\text{ g}$$

$$A^1A^2 = 2 + 4 = 6\text{ g}$$

$$A^2A^2 = 4 + 4 = 8\text{ g}$$

The genes that Nilsson-Ehle studied, which affected kernel color in wheat, were additive in this way.

Second, there is dominance genetic variance (V_D) when some genes have a dominance component. In this case, the alleles at a locus are not additive; rather, the effect of an allele depends on the identity of the other allele at that locus. Here, we cannot simply add the effects of the alleles together. Instead, we must add a component (V_D) to the genetic variance to account for the way that alleles interact. Third, genes at different loci may interact in the same way that alleles at the same locus interact. When this genic interaction occurs, the effects of genes are not additive, and we must include a third component, called genic interaction variance (V_I), to the genetic variance:

$$\mathbf{V_G = V_A + V_D + V_I}$$

Summary equation

We can now integrate these components into one equation to represent all the potential contributions to the phenotypic variance:

$$\mathbf{V_P = V_A + V_D + V_I + V_E + V_{GE}}$$

This equation provides us with a model that describes the potential causes of differences that we observe among individual phenotypes. It's important to note that this model deals strictly with the observable differences (variance) in phenotypes among individual members of a population; it says nothing about the absolute value of the characteristic or about the underlying genotypes that produce these differences.

Types of Heritability

The model of phenotypic variance that we've just developed can be used to address the question of how much of the phenotypic variance in a characteristic is due to genetic differences. Broad-sense heritability (H^2) represents the proportion of phenotypic variance that is due to genetic variance and is calculated by dividing the genetic variance by the phenotypic variance:

$$\text{broad-sense heritability} = H^2 = \frac{V_G}{V_P}$$

It is symbolized H^2 because it is a measure of variance, which is in units squared.

Broad-sense heritability can potentially range from 0 to 1. A value of 0 indicates that none of the phenotypic variance results from differences in genotype and all of the differences in phenotype result from environmental variation. A value of 1 indicates that all of the phenotypic variance results from differences in genotype. A heritability value between 0 and 1 indicates that both genetic and environmental factors influence the phenotypic variance.

Often, we are more interested in the proportion of the phenotypic variance that results from the additive genetic variance, because the additive genetic variance primarily determines the resemblance between parents and offspring.

Narrow-sense heritability (h^2) is equal to the additive genetic variance divided by the phenotypic variance:

$$\text{narrow-sense heritability} = h^2 = \frac{V_A}{V_P}$$

The Calculation of Heritability

Having considered the components that contribute to phenotypic variance and having developed a general concept of heritability, we can ask, How does one go about estimating these different components and calculating heritability?

There are several ways to measure the heritability of a characteristic. They include eliminating one or more variance components, comparing the resemblance of parents and offspring, comparing the phenotypic variances of individuals with different degrees of relatedness, and measuring the response to selection. The mathematical theory that underlies these calculations of heritability is complex and beyond the scope of this book. Nevertheless, we can develop a general understanding of how heritability is measured.

Heritability by elimination of variance components One way of calculating the broad-sense heritability is to eliminate one of the variance components. We have seen that $V_P = V_G + V_E + V_{GE}$. If we eliminate all environmental variance (VE = 0), then VGE = 0 (because, if either V_G or V_E is zero, no genetic–environmental interaction can take place), and $V_P = V_G$. In theory, we might make VE equal to 0 by ensuring that all individuals were raised in exactly the same environment but, in practice, it is virtually impossible. Instead, we could make V_G equal to 0 by raising genetically identical individuals, causing V_P to be equal to V_E. In a typical experiment, we might raise cloned or highly inbred, identically homozygous individuals in a defined environment and measure their phenotypic variance to estimate V_E.

We could then raise a group of genetically variable individuals and measure their phenotypic variance (VP). Using V_E calculated on the genetically identical individuals, we could obtain the genetic variance of the variable individuals by subtraction:

V_G[of genetically varying individuals]= V_P[of genetically varying individuals] - V_E[of genetically identical individuals]

The broad-sense heritability of the genetically variable individuals would then be calculated as follows:

$$H^2 = \frac{V_{G[\text{of genetically varying individuals}]}}{V_{P[\text{of genetically varying individuals}]}}$$

Sewall Wright used this method to estimate the heritability of white spotting in guinea pigs. He first measured the phenotypic variance for white spotting in a genetically variable population and found that $V_P = 573$. Then he inbred the guinea pigs for many generations so that they were essentially homozygous and genetically identical. When he measured their phenotypic variance in white spotting, he obtained V_P equal to 340. Because $V_G = 0$ in this group, their VP = VE. Wright assumed this value of environmental variance for the original (genetically variable) population and estimated their genetic variance:

$$V_P - V_E = V_G$$

$$573 - 340 = 233$$

He then estimated the broad-sense heritability from the genetic and phenotypic variance:

$$H^2 = \frac{V_G}{V_P}$$

$$H^2 = \frac{233}{573} = .41$$

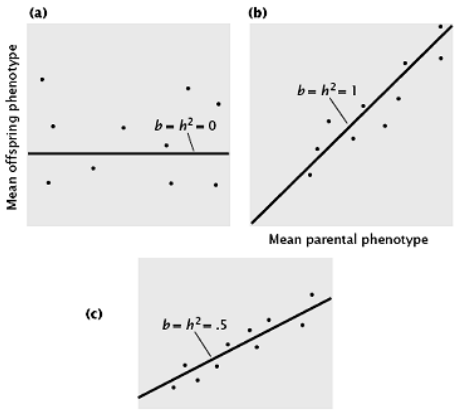
This value implies that 41% of the variation in spotting of guinea pigs in Wright's population was due to differences in genotype.

Estimating heritability by using this method assumes that the environmental variance of genetically identical individuals is the same as the environmental variance of the genetically variable individuals, which may not be true. Additionally, this approach can be applied only to organisms for which it is possible to create genetically identical individuals.

Heritability by parent–offspring regression Another method for estimating heritability is to compare the phenotypes of parents and offspring. When genetic differences are responsible for phenotypic variance, offspring should resemble their parents more than they resemble unrelated individuals, because offspring and parents have some genes in common that help determine their phenotype. Correlation and regression can be used to analyze the association of phenotypes in different individuals.

To calculate the narrow-sense heritability in this way, we first measure the characteristic on a series of parents and offspring. The data are arranged into families, and the mean parental phenotype is plotted against the mean offspring phenotype (FIGURE 6). Each data point in the graph represents one family; the value on the x (horizontal) axis is the mean phenotypic value of the parents in a family, and the value on the y (vertical) axis is the mean phenotypic value of the offspring for the family.

Let's assume that there is no narrow-sense heritability for the characteristic ($h^2 = 0$); genetic differences do not contribute to the phenotypic differences among individuals. In this case, offspring will be no more similar to their parents than they are to unrelated individuals, and the data points will be scattered randomly, generating a regression coefficient of zero (FIGURE 6a). Next, let's assume that all of the phenotypic differences are due to additive genetic differences ($h^2 = 1.0$). In this case, the mean phenotype of the offspring will be equal to the mean phenotype of the parents, and the regression coefficient will be 1 (FIGURE 6b). If genes and environment both contribute to the differences in phenotype, both heritability and the regression coefficient will lie between 0 and 1 (FIGURE 6c). The regression coefficient therefore provides information about the magnitude of the heritability.

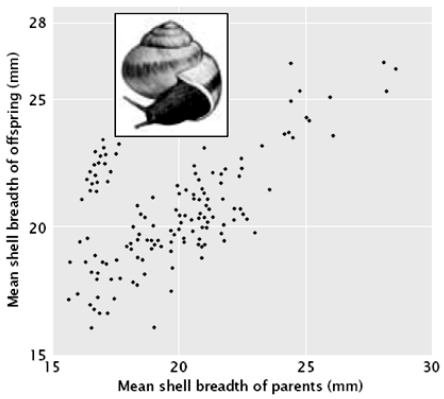


6 The narrow-sense heritability (h^2) equals the regression coefficient (b) in a regression of the mean phenotype of the offspring on the mean phenotype of the parents. (a) There is no relation between the parental phenotype and the offspring phenotype. (b) The offspring phenotype is the same as the parental phenotypes. (c) Both genes and environment contribute to the differences in phenotype.

A complex mathematical proof (which we will not go into here) demonstrates that, in a regression of the mean phenotype of the offspring against the mean phenotype of the parents, narrow-sense heritability (h^2) equals the regression coefficient (b):

$$h^2 = b_{(\text{regression of mean offspring against mean of both parents})}$$

An example of calculating heritability by regression of the phenotypes of parents and offspring is illustrated in FIGURE 7.



7 The heritability of shell breadth in snails can be determined by regression of the phenotype of offspring against the mean phenotype of the parents. The regression coefficient, which equals the heritability, is .70.

In a regression of the mean offspring phenotype against the phenotype of only one parent, the narrow-sense heritability equals twice the regression coefficient:

$$h^2 = 2b_{(\text{regression of mean offspring against mean of one parent})}$$

With only one parent, the heritability is twice the regression coefficient because only half the genes of the offspring come from one parent; thus, we must double the regression coefficient to obtain the full heritability.

Heritability and degrees of relatedness

A third method for calculating heritability is to compare the phenotypes of individuals having different degrees of relatedness.

This method is based on the concept that, the more closely related two individuals are, the more genes they have in common.

Monozygotic (identical) twins have 100% of their genes in common, whereas dizygotic (non-identical) twins have, on average, 50% of their genes in common. If genes are important in determining variability in a characteristic, then monozygotic twins should be more similar in a particular characteristic than dizygotic twins. By using correlation to compare the phenotypes of monozygotic and dizygotic twins, we can estimate broad-sense heritability. A rough estimate of the broad-sense heritability can be obtained by taking twice the difference of the correlation coefficients for a quantitative characteristic in monozygotic and dizygotic twins:

$$H^2 = 2(r_{\text{MZ}} - r_{\text{DZ}})$$

where r_{MZ} equals the correlation coefficient among monozygotic twins and r_{DZ} equals the correlation coefficient among dizygotic twins. This calculation assumes that the two individuals of a monozygotic twin pair experience environments that are no more similar to each other than those experienced by the two individuals of a dizygotic twin pair, which is often not the case, unless the twins have been reared apart. Narrow-sense heritability can also be estimated by comparing the phenotypic variances for a characteristic in full sibs (who have both parents in common, as well as 50% of their genes on the average) and half sibs (who have only one parent in common and thus 25% of their genes on the average).

All estimates of heritability depend on the assumption that the environments of related individuals are not more similar than those of unrelated individuals. This assumption is difficult to meet in human studies, because related people are usually reared together. Heritability estimates for humans should therefore always be viewed with caution.

The Limitations of Heritability

Knowledge of heritability has great practical value, because it allows us to statistically predict the phenotypes of offspring on the basis of their parent's phenotype. It also provides useful information about how characteristics will respond to selection (see next section). In spite of its importance, heritability is frequently misunderstood. Heritability does not provide information about an individual's genes or the environmental factors that control the development of a characteristic, and it says nothing about the nature of differences between groups. This section outlines some limitations and common misconceptions concerning broad- and narrow-sense heritability.

Heritability does not indicate the degree to which a characteristic is genetically determined Heritability is the proportion of the phenotypic variance that is due to genetic variance; it says nothing about the degree to which genes determine a characteristic. Heritability indicates only the degree to which genes determine variation in a characteristic. The determination of a characteristic and the determination of variation in a characteristic are two very different things.

Consider polydactyly (the presence of extra digits) in rabbits, which can be caused either by environmental factors or by a dominant gene. Suppose we have a group of rabbits all homozygous for a gene that produces normal numbers of digits. None of the rabbits in this group carries a gene for polydactyly, but a few of the rabbits are polydactylous because of environmental factors. Broad-sense heritability for polydactyly in this group is zero, because there is no genetic variation for polydactyly; all of the variation is due to environmental factors. However, it would be incorrect for us to conclude that genes play no role in determining the number of digits in rabbits. Indeed, we know that there are specific genes that can produce extra digits. Heritability indicates nothing about whether genes control the development of a characteristic; it only provides information about causes of the variation in a characteristic within a defined group.

An individual does not have heritability Broad- and narrow-sense heritabilities are statistical values based on the genetic and phenotypic variances found in a group of individuals. It is impossible to calculate heritability for an individual, and heritability has no meaning for a specific individual. Suppose we calculate the narrow-sense heritability of adult body weight for the students in a biology class and obtain a value of .6.We could conclude that 60% of the variation in adult body weight among the students in this class is determined by additive genetic variation. We could not, however, conclude that 60% of any particular student's body weight is due to additive genes.

There is no universal heritability for a characteristic The value of heritability for a characteristic is specific for a given population in a given environment. Recall that broad-sense heritability is genetic variance divided by phenotypic variance. Genetic variance depends on which genes are present, which often differs between populations. In the example of polydactyly in rabbits, there were no genes for polydactyly in the group; so the heritability of the characteristic was zero. A different group of rabbits might contain many genes for polydactyly, and the heritability of the characteristic might be high.

Environmental differences may affect heritability, because V_P is composed of both genetic and environmental variance.

When the environmental differences that affect a characteristic differ between two groups, the heritabilities for the two groups also will often differ. Because heritability is specific to a defined population in a given environment, it is important not to extrapolate heritabilities from one population to another. For example, human height is determined by environmental factors (such as nutrition and health) and by genes. If we measured the heritability of height in a developed country, we might obtain a value of .8, indicating that the variation in height in this population is largely genetic. This population has a high heritability because most people have adequate nutrition and health care (V_E is low); so most of the phenotypic variation in height is genetically determined. It would be incorrect for us to assume that height has a high heritability in all human populations. In developing countries, there may be more variation in a range of environmental factors; some people may enjoy good nutrition and health, whereas others may have a diet deficient in protein and suffer from diseases that affect stature. If we measured the heritability of height in such a country, we would undoubtedly obtain a lower value than we observed in the developed country, because there is more environmental variation and the genetic variance in height constitutes a smaller proportion of the phenotypic variation, making the heritability lower. The important point to remember is that heritability must be calculated separately for each population and each environment. Even when heritability is high, environmental factors may influence a characteristic High heritability does not mean that environmental factors cannot influence the expression of a characteristic. High heritability indicates only that the environmental variation to which the population is currently exposed is not responsible for variation in the characteristic.

Let's look again at human height. In most developed countries, heritability of human height is high, indicating that genetic differences are responsible for most of the variation in height. It would be wrong for us to conclude that human height cannot be changed by alteration of the environment. Indeed, height decreased in several European cities during World War II owing to hunger and disease, and height can be increased dramatically by the administration of growth hormone to children. The absence of environmental variation in a characteristic does not mean that the characteristic will not respond to environmental change.

Heritabilities indicate nothing about the nature of population differences in a characteristic A common misconception about heritability is that it provides information about population differences in a characteristic. Heritability is specific for a given population in a given environment, so it cannot be used to draw conclusions about why populations differ in a characteristic.

Suppose we measured heritability for human height in two groups. One group is from a small town in a developed country, where everyone consumes a high-protein diet. Because there is little variation in the environmental factors that affect human height and there is some genetic variation, the heritability of height in this group is high. The second group comprises the inhabitants of a single village in a developing country. The consumption of protein by these people is only 25% of that consumed by those in the first group; so their average adult height is several centimeters less than that in the developed country. Again, there is little variation in the environmental factors that determine height in this group, because everyone in the village eats the same types of food and is exposed to same diseases. Because there is little environmental variation and there is some genetic variation, the heritability of height in this group also is high. Thus, the heritability of height in both groups is high, and the average height in the two groups is considerably different. We might be tempted to conclude that the difference in height between the two groups is genetically based—that the people in the developed country are genetically taller than the people in the developing country. This conclusion is obviously wrong, however, because the differences in height are due largely to diet—an environmental factor. Heritability provides no information about the causes of differences between populations.

These limitations of heritability have often been ignored, particularly in arguments about possible social implications of genetic differences between humans. Soon after Mendel's principles of heredity were rediscovered, some geneticists began to claim that many human behavioral characteristics are determined entirely by genes. This claim led to debates about whether characteristics such as human intelligence are determined by genes or environment. Many of the early claims of genetically based human behavior were based on poor research; unfortunately, the results of these studies were often accepted at face value and led to a number of eugenic laws that discriminated against certain groups of people. Today, geneticists recognize that many behavioral characteristics are influenced by a complex interaction of genes and environment and that it is very difficult to separate genetic effects from those of the environment.

The results of a number of modern studies indicate that human intelligence as measured by IQ and other intelligence tests has a moderately high heritability (usually from .4 to .8). On the basis of this observation, some people have argued that intelligence is innate and that enhanced educational opportunities cannot boost intelligence. This argument is based on the misconception that, when heritability is high, changing the environment will not alter the characteristic. In addition, because heritabilities of intelligence range from .4 to .8, a considerable amount of the variance in intelligence originates from environmental differences.

Another argument based on a misconception about heritability is that ethnic differences in measures of intelligence are genetically based. Because the results of some genetic studies show that IQ has moderate heritability and because other studies find differences in the average IQ of ethnic groups, some people have suggested that ethnic differences in IQ are genetically based. As in the example of the effects of diet on nutrition, heritability provides no information about causes of differences among groups; it indicates only the degree to which phenotypic variance within a single group is genetically based. High heritability for a characteristic does not mean that phenotypic differences between ethnic groups are genetic. We should also remember that separating genetic and environmental effects in humans is very difficult; so heritability estimates themselves may be unreliable.

QTL MAPPING: LOCATING GENES THAT AFFECT QUANTITATIVE CHARACTERISTICS

The statistical methods described for use in analyzing quantitative characteristics can be used both to make predictions about the average phenotype expected in offspring and to estimate the overall contribution of genes to variation in the characteristic. These methods do not, however, allow us to identify and determine the influence of individual genes that affect quantitative characteristics. The genes that control polygenic characteristics are referred to as quantitative trait loci (QTLs). Although quantitative genetics has made important contributions to basic biology and to plant and animal breeding, the inability to identify QTLs and measure their individual effects has severely limited the application of quantitative genetic methods.

Mapping QTLs In recent years, numerous genetic markers have been identified and mapped with the use of recombinant DNA techniques, making it possible to identify QTLs by linkage analysis. The underlying idea is simple: if the inheritance of a genetic marker is associated consistently with the inheritance of a particular characteristic (such as increased height), then that marker must be linked to a QTL that affects height. The key is to have enough genetic markers so that QTLs can be detected throughout the genome. With the introduction of restriction fragment length polymorphisms and microsatellite variations, variable markers are now available for mapping QTLs in a number of different organisms.

A common procedure for mapping QTLs is to cross two homozygous strains that differ in alleles at many loci. The resulting F1 progeny are then intercrossed or backcrossed to allow the genes to recombine through independent assortment and crossing over.

Genes on different chromosomes and genes that are far apart on the same chromosome will recombine freely; genes that are closely linked will be inherited together. The offspring are measured for one or more quantitative characteristics; at the same time, they are genotyped for numerous genetic markers that span the genome. Any correlation between the inheritance of a particular marker allele and a quantitative phenotype indicates that a QTL is linked to that marker. If enough markers are used, it is theoretically possible to detect all the QTLs affecting a characteristic. This approach has been used to detect genes affecting various characteristics in several plant and animal species (Table 2).

Applications of QTL mapping The number of genes affecting a quantitative characteristic can be estimated by locating QTLs with genetic markers and adding up the number of QTLs detected. This method will always be an underestimate, because QTLs that are located close together on the same chromosome will be counted together, and those with small effects are likely to be missed.

Table 2 Quantitative characteristics for which QTLs have been detected

Organism	Quantitative Characteristic	Number of QTLs Detected
Tomato	Soluble solids	7
	Fruit mass	13
	Fruit pH	9
	Growth	5
	Leaflet shape	9
	Height	9
Corn	Height	11
	Leaf length	7
	Tiller number	1
	Glume hardness	5
	Grain yield	18
	Number of ears	9
	Thermotolerance	6
Common bean	Number of nodules	4
Mung bean	Seed weight	4
Cow pea	Seed weight	2
Wheat	Preharvest sprout	4
Pig	Growth	2
	Length of small intestine	1
	Average back fat	1
	Abdominal fat	1
Mouse	Epilepsy	2
Rat	Hypertension	2

QTL mapping also provides information about the magnitude of the effects that individual genes have on a quantitative characteristic. The polygenic model assumes that many genes affect a quantitative characteristic, that the effect of each gene is small, and that the effects of the genes are equal and additive. The results of studies of QTLs in a number of organisms now show that these assumptions are not always valid. Polygenes appear to vary widely in their effects. In many of the characteristics that have been studied, a few QTLs account for much of the phenotypic variation.

In some instances, individual QTLs have been mapped that account for more than 20% of the variance in the characteristic.

I. Mutation: Types, causes and detection, mutant types – lethal, conditional, biochemical, loss of function, gain of function, germinal verses somatic mutants, insertional mutagenesis.

The Nature of Mutation

DNA is a highly stable molecule that replicates with amazing accuracy, but changes in DNA structure and errors of replication do occur. A mutation is defined as an inherited change in genetic information; the descendants may be cells produced by cell division or individual organisms produced by reproduction.

The Importance of Mutations

Mutations are both the sustainer of life and the cause of great suffering. On the one hand, mutation is the source of all genetic variation, the raw material of evolution. Without mutations and the variation that they generate, organisms could not adapt to changing environments and would risk extinction. On the other hand, most mutations have detrimental effects, and mutation is the source of many human diseases and disorders.

Much of genetics focuses on how variants produced by mutation are inherited; genetic crosses are meaningless if all individuals are identically homozygous for the same alleles. Mutations serve as important tools of genetic analysis; the solution to almost any genetic problem begins with a good set of mutants. Much of Gregor Mendel's success in unraveling the principles of inheritance can be traced to his use of carefully selected variants of the garden pea; similarly, Thomas Hunt Morgan and his students discovered many basic principles of genetics by analyzing mutant fruit flies.

Mutations are also useful for probing fundamental biological processes. Finding mutations that affect different components of a biological system and studying their effects can often lead to an understanding of the system. This method, referred to as genetic dissection, is analogous to figuring out how an automobile works by breaking different parts of a car and observing the effects—for example, smash the radiator and the engine overheats, revealing that the radiator cools the engine. The disruption of function in individual organisms bearing particular mutations likewise can be a source of insight into biological processes. For example, geneticists have begun to unravel the molecular details of development by studying mutations that interrupt various embryonic stages in Drosophila. Although this method of breaking "parts" to determine their function might seem like a crude approach to understanding a system, it is actually very powerful and has been used extensively in biochemistry, developmental biology, physiology, and behavioral science (but this method is not recommended for learning how your car works).

Categories of Mutations

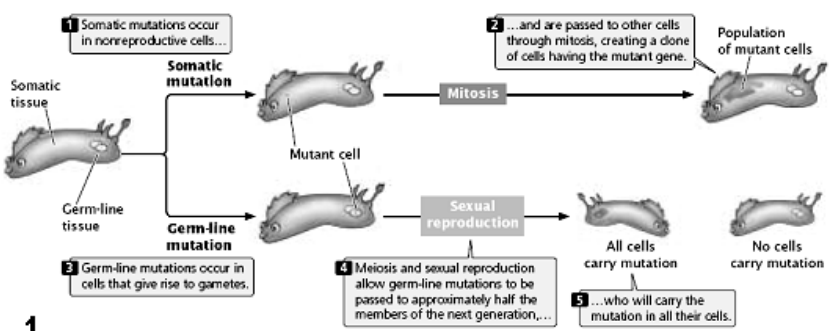
In multicellular organisms, we can distinguish between two broad categories of mutations: somatic mutations and germline mutations. Somatic mutations arise in somatic tissues, which do not produce gametes (FIGURE 1). These mutations are passed on to other cells through the process of mitosis, which leads to a population of genetically identical cells (a clone). The earlier in development that a somatic mutation occurs, the larger the clone of cells within that individual organism that will contain the mutation.

Because of the huge number of cells present in a typical eukaryotic organism, **somatic mutations** must be numerous. For example, there are about 10^{14} cells in the human body. If a mutation arises only once in every million cell divisions (a fairly typical rate of mutation), hundreds of millions of somatic mutations must arise in each person.

The effect of these mutations depends on many factors, including the type of cell in which they occur and the developmental stage at which they arise. Many somatic mutations have no obvious effect on the phenotype of the organism, because the function of the mutant cell (even the cell itself) is replaced by that of normal cells. However, cells with a somatic mutation that stimulates cell division can increase in number and spread; this type of mutation can give rise to cells with a selective advantage and is the basis for all cancers.

Germ-line mutations arise in cells that ultimately produce gametes. These mutations can be passed to future generations, producing individual organisms that carry the mutation in all their somatic and germ-line cells (see Figure 1). When we speak of mutations in multicellular organisms, we're usually talking about germ-line mutations. In single-cell organisms, however, there is no distinction between germ-line and somatic mutations, because cell division results in new individuals.

Historically, mutations have been partitioned into those that affect a single gene, called gene mutations, and those that affect the number or structure of chromosomes, called chromosome mutations. This distinction arose because



1 There are two basic classes of mutations: somatic mutations and germ-line mutations.

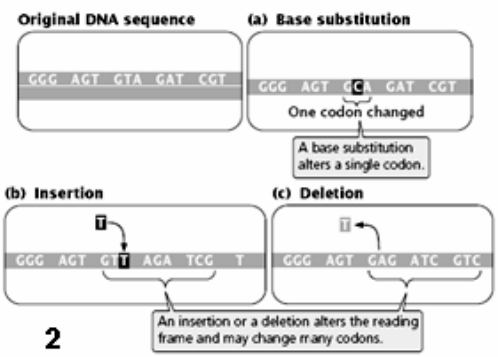
chromosome mutations could be observed directly, by looking at chromosomes with a microscope, whereas gene mutations could be detected only by observing their phenotypic effects. Now, with the development of DNA sequencing, gene mutations and chromosome mutations are distinguished somewhat arbitrarily on the basis of the size of the DNA lesion. Nevertheless, it is useful to use the term chromosome mutation for a large-scale genetic alteration that affects chromosome structure or the number of chromosomes and the term gene mutation for a relatively small DNA lesion that affects a single gene. This topic focuses on gene mutations.

Types of Gene Mutations

There are a number of ways to classify gene mutations. Some classification schemes are based on the nature of the phenotypic effect—whether the mutation alters the amino acid sequence of the protein and, if so, how. Other schemes are based on the causative agent of the mutation, and still others focus on the molecular nature of the defect. The most appropriate scheme depends on the reason for studying the mutation. Here, we will categorize mutations primarily on the basis of their molecular nature, but we will also encounter some terms that relate the causes and the phenotypic effects of mutations.

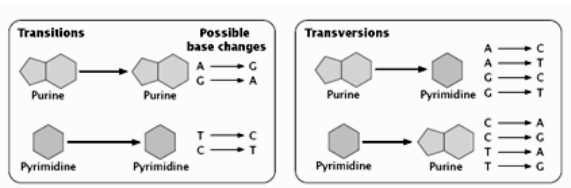
Base substitutions

The simplest type of gene mutation is a base substitution, the alternation of a single nucleotide in the DNA (FIGURE 2a). Because of the complementary nature of the two DNA strands, when the base of one nucleotide is altered, the base of the corresponding nucleotide on the opposite strand also will be altered in the next round of replication. A base substitution therefore usually leads to a base-pair substitution.



Three basic types of gene mutations are base substitutions, insertions, and deletions.

Base substitutions are of two types. In a transition, a purine is replaced by a different purine or, alternatively, a pyrimidine is replaced by a different pyrimidine (FIGURE 3). In a transversion, a purine is replaced by a pyrimidine or a pyrimidine is replaced by a purine. The number of possible transversions is twice the number of possible transitions, but transitions usually arise more frequently.



A transition is the substitution of a purine for a purine or a pyrimidine for a pyrimidine; a transversion is the substitution of a pyrimidine for a purine or a purine for a pyrimidine.

Insertions and deletions

The second major class of gene mutations contains insertions and deletions—the addition or the removal, respectively, of one or more nucleotide pairs (FIGURE 2b and c). Although base substitutions are often assumed to be the most common type of mutation, molecular analysis has revealed that insertions and deletions are more frequent. Insertions and deletions within sequences that encode proteins may lead to frameshift mutations, changes in the reading frame of the gene. The initiation codon in mRNA sets the reading frame: after the initiation codon, other codons are read as successive non-overlapping groups of three nucleotides. The addition or deletion of a nucleotide usually changes the reading frame, altering all amino acids encoded by codons following the mutation (see Figure 2 b and c).

Many amino acids can be affected; so frameshift mutations generally have drastic effects on the phenotype. Not all insertions and deletions lead to frameshifts, however; because codons consist of three nucleotides, insertions and deletions consisting of any multiple of three nucleotides will leave the reading frame intact, although the addition or removal of one or more amino acids may still affect the phenotype. These mutations are called in-frame insertions and deletions, respectively.

Expanding trinucleotide repeats In 1991, an entirely novel type of mutation was discovered. This mutation occurs in a gene called FMR-1 and causes fragile-X syndrome, the most common hereditary cause of mental retardation. The disorder is so named because, in specially treated cells of persons having the condition, the tip of the X chromosome is attached only by a slender thread. The FMR-1 gene contains a number of adjacent copies of the trinucleotide CGG. The normal FMR-1 allele (not containing the mutation) has 60 or fewer copies of this trinucleotide but, in persons with fragile-X syndrome, the allele may harbor hundreds or even thousands of copies. Mutations in which copies of a trinucleotide may increase greatly in number are called expanding trinucleotide repeats.

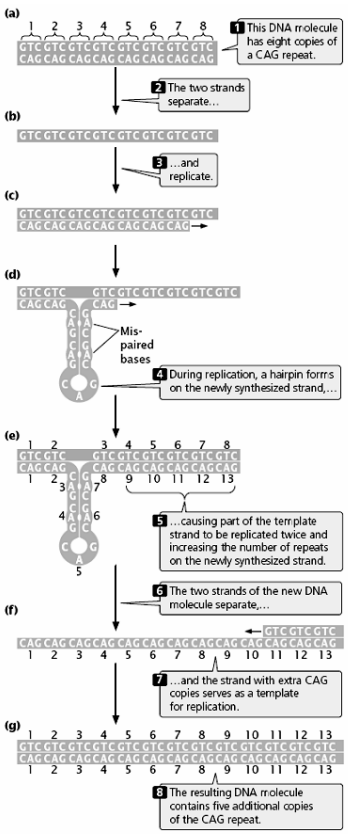
Expanding trinucleotide repeats have been found in several other human diseases (Table 1). The number of copies of the trinucleotide repeat often correlates with the severity or age of onset of the disease. The number of copies of the repeat also correlates with the instability of trinucleotide repeats—when more repeats are present, the probability of expansion to even more repeats increases. This instability leads to a phenomenon known as anticipation, in which diseases caused by trinucleotide-repeat expansions become more severe in each generation. Less commonly, the number of trinucleotide repeats may decrease within a family.

Table 1 Examples of genetic diseases caused by expanding trinucleotide repeats

Disease	Repeated Sequence	Number of Copies of Repeat: Normal Range	Number of Copies of Repeat: Disease Range
Spinal and bulbar muscular atrophy	CAG	11–33	40–62
Fragile-X syndrome	CGG	6–54	50–1500
Jacobsen syndrome	CGG	11	100–1000
Spinocerebellar ataxia (several types)	CAG	4–44	21–130
Autosomal dominant cerebellar ataxia	CAG	7–19	37–~220
Myotonic dystrophy	CTG	5–37	44–3000
Huntington disease	CAG	9–37	37–121
Friedreich ataxia	GAA	6–29	200–900
Dentatorubral-pallidoluysian atrophy	CAG	7–25	49–75
Myoclonus epilepsy of the Unverricht-Lundborg type*	CCCGCCCGCG	2–3	12–13

*Technically not a trinucleotide repeat but does entail a multiple of three nucleotides that expands and contracts in similar fashion to trinucleotide repeats.

How an increase in the number of trinucleotides produces disease symptoms is not yet clear. In several of the diseases (e.g., Huntington disease), the trinucleotide CAG expands within the coding part of a gene, producing a toxic protein that has extra glutamine residues (the amino acid encoded by CAG). In other diseases (e.g., fragile-X syndrome and myotonic dystrophy), the repeat is outside the coding region of the gene and therefore must have some other mode of action. At least one disease (a rare type of epilepsy) has now been associated with an expanding repeat of a 12-bp sequence. Although this repeat is not a trinucleotide, it is included as a type of expanding trinucleotide because its repeat is a multiple of three.



4 The number of copies of a trinucleotide may increase by strand slippage in replication.

The mechanism that leads to the expansion of trinucleotide repeats is still unclear. Strand slippage in DNA replication and crossing over between misaligned repeats are two possible sources of expansion. Single-stranded regions of some trinucleotide repeats are known to fold into hairpins (FIGURE 4) and other special DNA structures. Such structures may promote strand slippage in replication and may prevent these errors from being recognized and corrected.

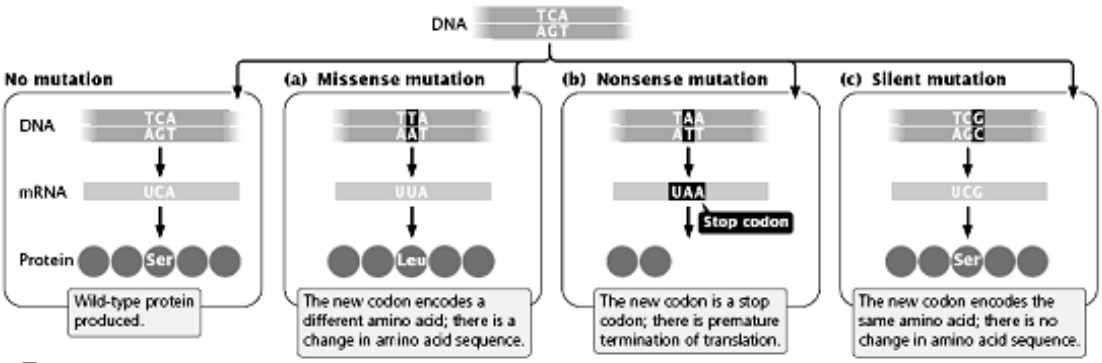
Phenotypic effects of mutations

Mutations have a variety of phenotypic effects. The effect of a mutation must be considered with reference to a phenotype against which the mutant can be compared, which is usually the wild-type phenotype—that is, the most common phenotype in natural populations of the organism. For example, most *Drosophila melanogaster* in nature have red eyes; so red eyes are considered the wild-type eye color; any other genetically determined eye color in fruit flies is considered to be a mutant. A mutation that alters the wild-type phenotype is called a forward mutation, where as a reverse mutation (a reversion) changes a mutant phenotype back into the wild type.

Geneticists use special terms to describe the phenotypic effects of mutations. A base substitution that alters a codon in the mRNA, resulting in a different amino acid in the protein, is referred to as a missense mutation(FIGURE 5a). A nonsense mutation changes a sense codon (one that specifies an amino acid) into a nonsense codon (one that terminates translation; FIGURE 5b). If a nonsense mutation occurs early in the mRNA sequence, the protein will be greatly shortened and will usually be nonfunctional.

A silent mutation alters a codon but, thanks to the redundancy of the genetic code, the codon still specifies the same amino acid (FIGURE 5c). A neutral mutation is a missense mutation that alters the amino acid sequence of the protein but does not change its function. Neutral mutations occur when one amino acid is replaced by another that is chemically similar or when the affected amino acid has little influence on protein function.

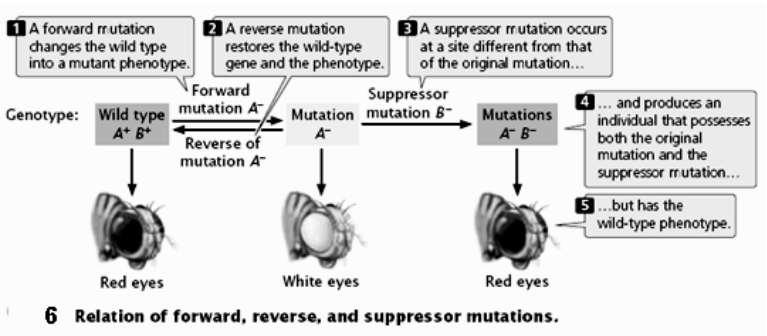
Loss-of-function mutations cause the complete or partial absence of normal function. A loss-of-function mutation so alters the structure of the protein that the protein no longer works correctly or the mutation can occur in regulatory regions that affect the transcription, translation, or splicing of the protein. Loss-of-function mutations are frequently recessive, and diploid individuals must be homozygous for the mutation before they can exhibit the effects of the loss of the functional protein. In contrast, a gain-of-function mutation produces an entirely new trait or it causes a trait to appear in inappropriate tissues or at inappropriate times in development. These mutations are frequently dominant in their expression. Still other types of mutations are conditional mutations, which are expressed only under certain conditions, and lethal mutations, which cause premature death.



5 **Base substitutions can cause (a) missense, (b) nonsense, and (c) silent mutations.**

Suppressor mutations

A suppressor mutation is a genetic change that hides or suppresses the effect of another mutation. This type of mutation is distinct from a reverse mutation, in which the mutated site changes back into the original wild-type sequence (FIGURE 6). A suppressor mutation occurs at a site that is distinct from the site of the original mutation; thus, an individual organism with a suppressor mutation is a double mutant, possessing both the original mutation and the suppressor mutation but exhibiting the phenotype of an unmutated wild type.



6 **Relation of forward, reverse, and suppressor mutations.**

Geneticists distinguish between two classes of suppressor mutations: intragenic and intergenic. An intragenic suppressor is in the same gene as that containing the mutation being suppressed and may work in several ways. The suppressor may change a second nucleotide in the same codon that was altered by the original mutation, producing a codon that specifies the same amino acid as the original, unmutated codon (FIGURE 7). Intragenic suppressors may also work by suppressing a frameshift mutation. If the original mutation is a one-base deletion, then the addition of a single base elsewhere in the gene will restore the former reading frame (see Figure 7). Consider the following nucleotide sequence in DNA and the amino acids that it encodes:

DNA	AAA	TCA	CTT	GGC	GTA	CAA
Amino acids	Phe	Ser	Glu	Pro	His	Val

Suppose a one-base deletion occurs in the first nucleotide of the second codon. This deletion shifts the reading frame by one nucleotide and alters all the amino acids that follow the mutation.

One-nucleotide deletion

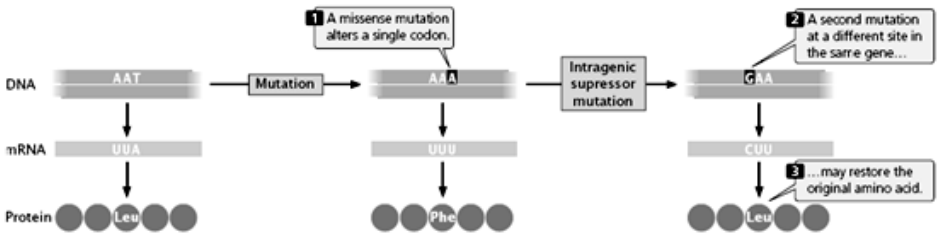
AAA	XCAC	TTG	GCG	TAC	AA
Phe	Val	Asn	Arg	Stop	

If a single nucleotide is added to the third codon (the suppressor mutation), the reading frame is restored, although two of the amino acids differ from those specified by the original sequence.

One-nucleotide duplication

AAA	CAC	TTX	GGC	GTA	CAA
Phe	Val	Lys	Pro	His	Val

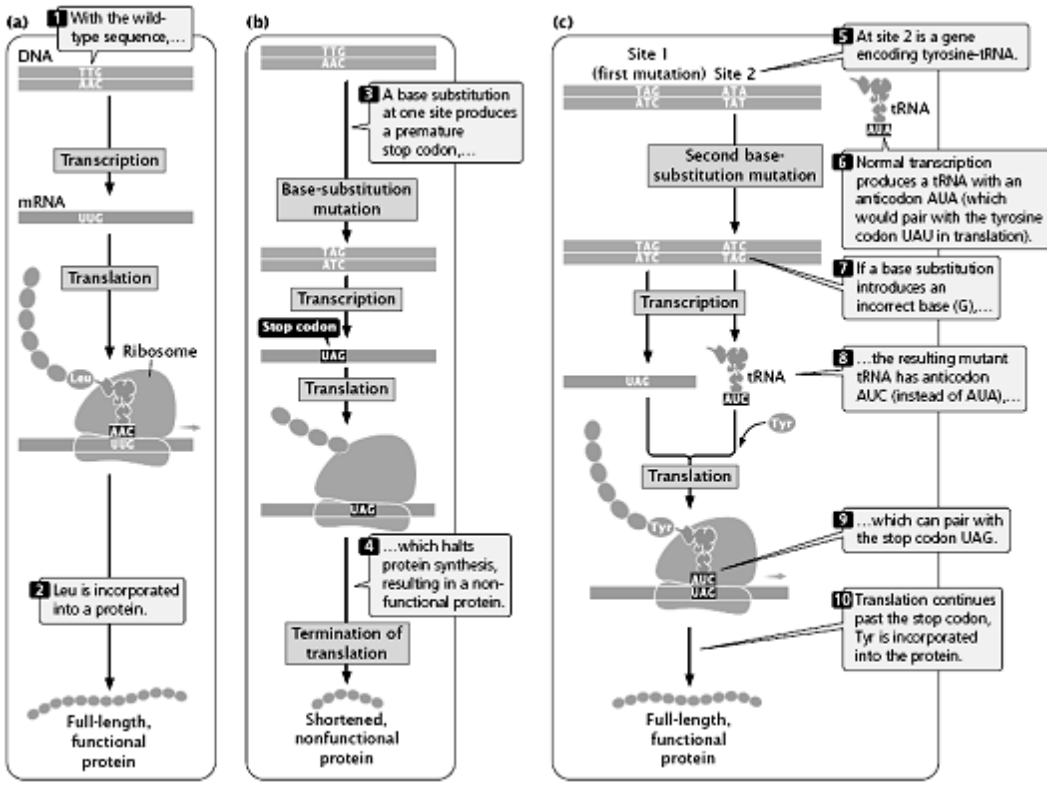
Similarly, a mutation due to an insertion may be suppressed by a subsequent deletion in the same gene. A third way in which an intragenic suppressor may work is by making compensatory changes in the protein. A first missense mutation may alter the folding of a polypeptide chain by changing the way in which amino acids in the protein interact with one another. A second missense mutation at a different site (the suppressor) may recreate the original folding pattern by restoring interactions between the amino acids.



7 **An intragenic suppressor mutation occurs in the same gene that contains the mutation being suppressed.**

Intergenic suppressors, in contrast, occur in a gene that is different from the one bearing the original mutation. These suppressors sometimes work by changing the way that the mRNA is translated. In the example illustrated in (FIGURE 8), the original DNA sequence is AAC (UUG in the mRNA)

and specifies leucine. This sequence mutates to ATC (UAG in mRNA), a termination codon. The ATC nonsense mutation could be suppressed by a mutation in a gene that encodes a tRNA molecule by changing the anticodon on the tRNA so that it is capable of pairing with the UAG termination codon. For example, the gene that encodes the tRNA for tyrosine ($tRNA^{Tyr}$), which has the anticodon AUA, might be mutated to have the anticodon AUC, which will then pair with the UAG stop codon. Instead of translation terminating at the UAG codon, tyrosine would be inserted into the protein and a full-length protein would be produced, although tyrosine would now substitute for leucine. The effect of this change would depend on the role of this amino acid in the overall structure of the protein, but the effect is likely to be less detrimental than the effect of the nonsense mutation, which would halt translation prematurely.



8 An intergenic suppressor mutation occurs in a different gene from the one bearing the original mutation.

Because cells in many organisms have multiple copies of tRNA genes, other unmutated copies of $tRNA^{Tyr}$ would remain available to recognize the tyrosine codons. However, we might expect that the tRNAs that have undergone a suppressor mutation would also suppress the normal termination codons at the ends of coding sequences, resulting in the production of longer-than-normal proteins, but this event does not usually take place. Mutations in tRNA genes can also suppress missense and frameshift mutations. Intergenic suppressors can also work through genic interactions. Polypeptide chains which are produced by two genes may interact to produce a functional protein. A mutation in one gene may alter the encoded polypeptide so that the interaction is destroyed and then a functional protein is no longer produced.

A suppressor mutation in the second gene may produce a compensatory change in its polypeptide therefore restoring the original interaction. Characteristics of some of the different types of mutations are summarized in Table 2.

Mutation Rates

The frequency with which a gene changes from the wild type to a mutant is referred to as the mutation rate and is generally expressed as the number of mutations per biological unit, which may be mutations per cell division, per gamete, or per round of replication. For example, the mutation rate for achondroplasia (a type of hereditary dwarfism) is about four mutations per 100,000 gametes, usually expressed more simply as 4×10^{5}. In contrast, mutation frequency is defined as the incidence of a specific type of mutation within a group of individual organisms.

For achondroplasia, the mutation frequency in the United States is about 2×10^{4}, which means that about 1 of every 20,000 persons in the U.S. population carries this mutation.

Mutation rates are affected by three factors. First, they depend on the frequency with which primary changes take place in DNA. Primary change may arise from spontaneous molecular changes in DNA or it may be induced by chemical or physical agents in the environment. A second factor influencing the mutation rate is the probability that, when a

change takes place, it will be repaired. Most cells possess a number of mechanisms to repair altered DNA; so most alterations are corrected before they are replicated. If these repair systems are effective, mutation rates will be low; if they are faulty, mutation rates will be elevated. Some mutations increase the overall rate of mutation at other genes; these mutations usually occur in genes that encode components of the replication machinery or DNA repair enzymes.

Table 2 Characteristics of different types of mutations

Type of Mutation	Definition
Base substitution	Changes the base of a single DNA nucleotide
Transition	Base substitution in which a purine replaces a purine or a pyrimidine replaces a pyrimidine
Transversion	Base substitution in which a purine replaces a pyrimidine or a pyrimidine replaces a purine
Insertion	Addition of one or more nucleotides
Deletion	Deletion of one or more nucleotides
Frameshift mutation	Insertion or deletion that alters the reading frame of a gene
In-frame deletion or insertion	Insertion or deletion of a multiple of three nucleotides that does not alter the reading frame
Expanding trinucleotide repeats	Repeated sequence of three nucleotides (trinucleotide) in which the number of copies of the trinucleotide increases
Forward mutation	Changes the wild-type phenotype to a mutant phenotype
Reverse mutation	Changes a mutant phenotype back to the wild-type phenotype
Missense mutation	Changes a sense codon into a different sense codon, resulting in the incorporation of a different amino acid in the protein
Nonsense mutation	Changes a sense codon into a nonsense codon, causing premature termination of translation
Silent mutation	Changes a sense codon into a synonymous codon, leaving unchanged the amino acid sequence of the protein
Neutral mutation	Changes the amino acid sequence of a protein without altering its ability to function
Loss-of-function mutation	Causes a complete or partial loss of function
Gain-of-function mutation	Causes the appearance of a new trait or function or causes the appearance of a trait in inappropriate tissues or at inappropriate times
Lethal mutation	Causes premature death
Suppressor mutation	Suppresses the effect of an earlier mutation at a different site
Intragenic suppressor mutation	Suppresses the effect of an earlier mutation within the same gene
Intergenic suppressor mutation	Suppresses the effect of an earlier mutation in another gene

A third factor, one that influences our ability to calculate mutation rates, is the probability that a mutation will be recognized and recorded. When DNA is sequenced, all mutations are potentially detectable. In practice, however, sequencing is expensive; so mutations are usually detected by their phenotypic effects. Some mutations may appear to arise at a higher rate simply because they are easier to detect.

Mutation rates vary among organisms and among genes within organisms (Table 3), but we can draw several general conclusions about mutation rates. First, spontaneous mutation rates are low for all organisms studied. Typical mutation rates for viral and bacterial genes range from about 1 to 100 mutations per 10 billion cells (1×10^8 to 1×10^{10}). The mutation rates for most eukaryotic genes are a bit higher, from about 1 to 10 mutations per million gametes (1×10^5 to 1×10^6). These higher values in eukaryotes may be due to the fact that the rates are calculated per gamete, and several cell divisions are required to produce a gamete, whereas mutation rates in prokaryotic cells and viruses are calculated per cell division.

Table 3 Mutation rates of different genes in different organisms

Organism	Mutation	Rate	Unit
Bacteriophage T2	Lysis inhibition	1×10^{-8}	Per replication
	Host range	3×10^{-9}	
Escherichia coli	Lactose fermentation	2×10^{-7}	Per cell division
	Histidine requirement	2×10^{-8}	
Neurospora crassa	Inositol requirement	8×10^{-8}	Per asexual spore
	Adenine requirement	4×10^{-8}	
Corn	Kernel color	2.2×10^{-6}	Per gamete
Drosophila	Eye color	4×10^{-5}	Per gamete
	Allozymes	5.14×10^{-6}	
Mouse	Albino coat color	4.5×10^{-5}	Per gamete
	Dilution coat color	3×10^{-5}	
Human	Huntington disease	1×10^{-6}	Per gamete
	Achondroplasia	1×10^{-5}	
	Neurofibromatosis (Michigan)	1×10^{-4}	
	Hemophilia A (Finland)	3.2×10^{-5}	
	Duchenne muscular dystrophy (Wisconsin)	9.2×10^{-5}	

Within each major class of organisms, mutation rates vary considerably. These differences may be due to differing abilities to repair mutations, unequal exposures to mutagens, or biological differences in rates of spontaneously arising mutations. Even within a single species, spontaneous rates of mutation vary among genes. The reason for this variation is not entirely understood, but some regions of DNA are known to be more susceptible to mutation than others.

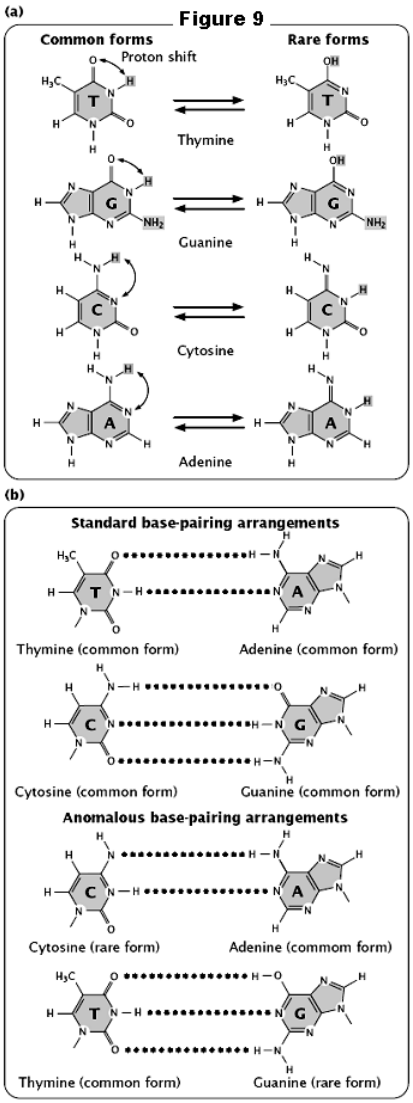
Causes of Mutations

Mutations result from both internal and external factors. Those that are a result of natural changes in DNA structure are termed spontaneous mutations, whereas those that result from changes caused by environmental chemicals or radiation are induced mutations.

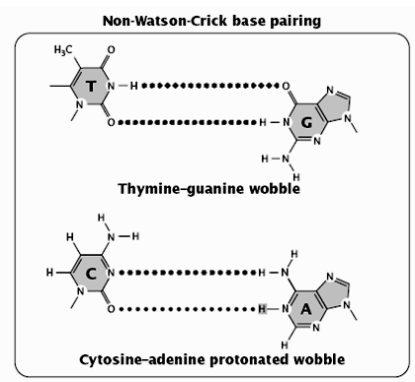
Spontaneous Replication Errors

Replication is amazingly accurate: fewer than one in a billion errors are made in the course of DNA synthesis. However, spontaneous replication errors do occasionally occur.

The primary cause of spontaneous replication errors was formerly thought to be tautomeric shifts, in which the positions of protons in the DNA bases change. Purine and pyrimidine bases exist in different chemical forms called tautomers (FIGURE 9a). The two tautomeric forms of each base are in dynamic equilibrium, although one form is more common than the other. The standard Watson and Crick base pairings—adenine with thymine, and cytosine with guanine—are between the common forms of the bases, but, if the bases are in their rare tautomeric forms, other base pairings are possible (FIGURE 9b). Watson and Crick proposed that tautomeric shifts might produce mutations, and for many years their proposal was the accepted model for spontaneous replication errors, but there has never been convincing evidence that the rare tautomers are the cause of spontaneous mutations. Furthermore, research now shows little evidence of these structures in DNA.

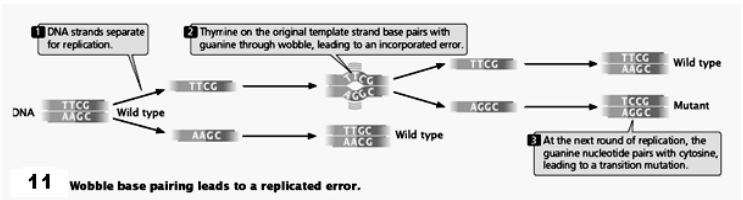


Mispairing can also occur through wobble, in which normal, protonated, and other forms of the bases are able to pair because of flexibility in the DNA helical structure (FIGURE 10).



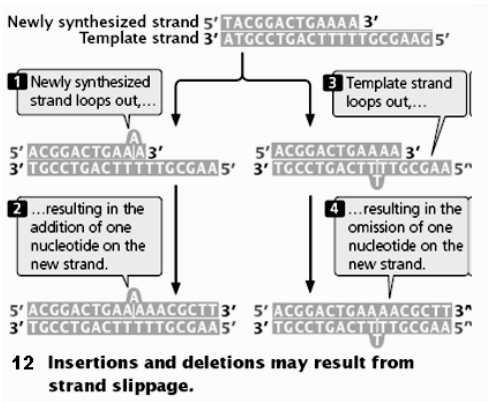
10 Nonstandard base pairings can occur as a result of the flexibility in DNA structure. Thymine and guanine can pair through wobble between normal bases. Cytosine and adenine can pair through wobble when adenine is protonated (has an extra hydrogen).

These structures have been detected in DNA molecules and are now thought to be responsible for many of the mispairings in replication. When a mismatched base has been incorporated into a newly synthesized nucleotide chain, an incorporated error is said to have occurred. Suppose that, in replication, thymine (which normally pairs with adenine) mispairs with guanine through wobble (FIGURE 11). In the next round of replication, the two mismatched bases separate, and each serves as template for the synthesis of a new nucleotide strand. This time, thymine pairs with adenine, producing another copy of the original DNA sequence. On the other strand, however, the incorrectly incorporated guanine serves as the template and pairs with cytosine, producing a new DNA molecule that has an incorporated error—a C.G pair in place of the original T.A pair (a T.A→C.G base substitution). The original incorporated error leads to a replication error, which creates a permanent mutation, because all the base pairings are correct and there is no mechanism for repair systems to detect the error.



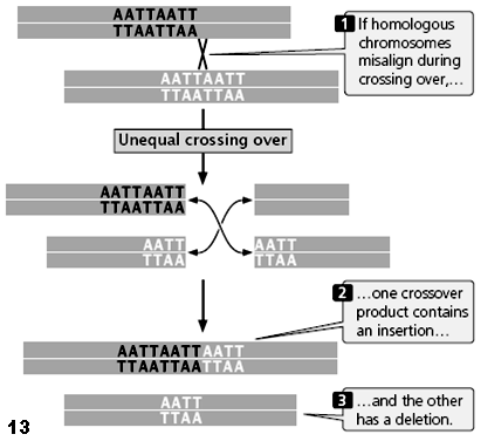
11 Wobble base pairing leads to a replicated error.

Mutations due to small insertions and deletions also may arise spontaneously in replication and crossing over. Strand slippage may occur when one nucleotide strand forms a small loop (FIGURE 12).



12 Insertions and deletions may result from strand slippage.

If the looped-out nucleotides are on the newly synthesized strand, an insertion results. At the next round of replication, the insertion will be incorporated into both strands of the DNA molecule. If the looped-out nucleotides are on the template strand, then there is a deletion on the newly replicated strand, and this deletion will be perpetuated in subsequent rounds of replication. During normal crossing over, the homologous sequences of the two DNA molecules align, and crossing over produces no net change in the number of nucleotides in either molecule. Misaligned pairing may cause unequal crossing over, which results in one DNA molecule with an insertion and the other with a deletion (FIGURE 13).



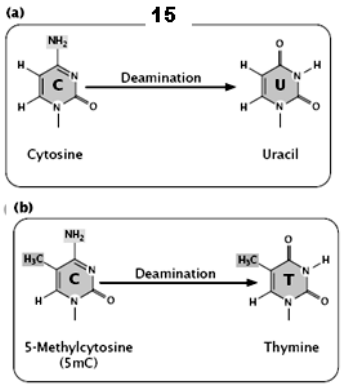
Unequal crossing over produces insertions and deletions.

Some DNA sequences are more likely than others to undergo strand slippage or unequal crossing over. Stretches of repeated sequences, such as trinucleotide repeats or homopolymeric repeats (more than five repeats of the same base in a row), are prone to strand slippage. Stretches with more repeats are more likely to undergo strand slippage. Duplicated or repetitive sequences may misalign during pairing, leading to unequal crossing over. Both strand slippage and unequal crossing over produce duplicated copies of sequences, which in turn promote further strand slippage and unequal crossing over. This chain of events may explain the phenomenon of anticipation often observed for expanding trinucleotide repeats.

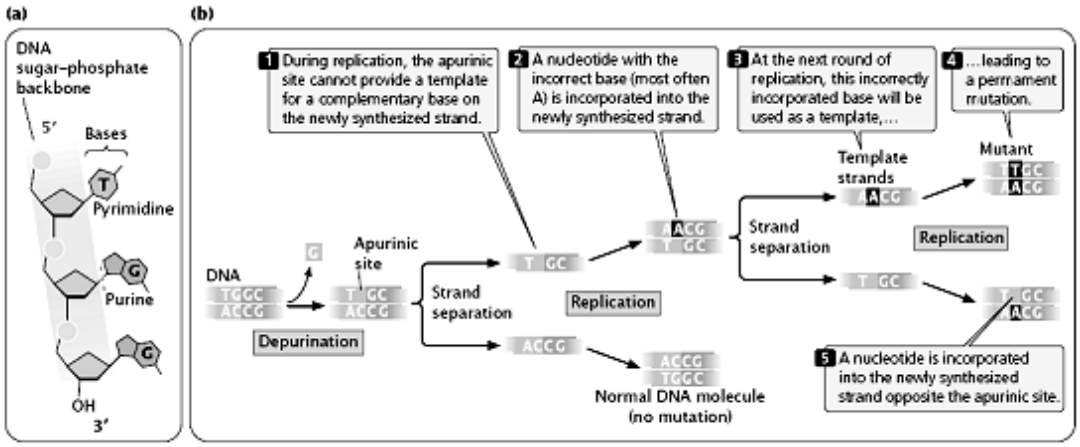
Spontaneous Chemical Changes

In addition to spontaneous mutations that arise in replication, mutations also result from spontaneous chemical changes in DNA. One such change is depurination, the loss of a purine base from a nucleotide. Depurination results when the covalent bond connecting the purine to the 1'-carbon atom of the deoxyribose sugar breaks (FIGURE 14a), producing an apurinic site—a nucleotide that lacks its purine base. An apurinic site cannot act as a template for a complementary base in replication. In the absence of base-pairing constraints, an incorrect nucleotide (most often adenine) is incorporated into the newly synthesized DNA strand opposite the apurinic site(FIGURE 14b), frequently leading to an incorporated error. The incorporated error is then transformed into a replication error at the next round of replication. Depurination is a common cause of spontaneous mutation; a mammalian cell in culture loses approximately 10,000 purines every day.

Another spontaneously occurring chemical change that takes place in DNA is deamination, the loss of an amino group (NH_2) from a base. Deamination may occur spontaneously or be induced by mutagenic chemicals.



Deamination may alter the pairing properties of a base: the deamination of cytosine, for example, produces uracil (FIGURE 15a), which pairs with adenine during replication. After another round of replication, the adenine will pair with thymine, creating a T.A pair in place of the original C.G pair (C.G→U.A→T.A); this chemical change is a transition mutation. This type of mutation is usually repaired by enzymes that remove uracil whenever it is found in DNA. The ability to recognize the product of cytosine deamination may explain why thymine, not uracil, is found in DNA. Some cytosine bases in DNA are naturally methylated and exist in the form of 5-methylcytosine (5mC) which when deaminated becomes thymine (FIGURE 15b). Because thymine pairs with adenine in replication, the deamination of 5-methylcytosine changes an original C.G pair to T.A (C.G→5mC.A→T.A). This change cannot be detected by DNA repair systems, because it produces a normal base. Consequently, C.G→T.A transitions occur frequently in eukaryotic cells.



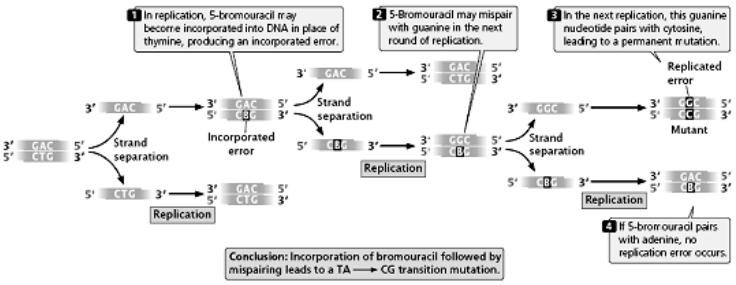
Depurination, loss of a purine base from the nucleotide, produces an apurinic site.

Chemically Induced Mutations

Although many mutations arise spontaneously, a number of environmental agents are capable of damaging DNA, including certain chemicals and radiation. Any environmental agent that significantly increases the rate of mutation above the spontaneous rate is called a mutagen. The first discovery of a chemical mutagen was made by Charlotte Auerbach, who was born in Germany to a Jewish family in 1899. After attending university in Berlin and doing research, she spent several years teaching at various schools in Berlin. Faced with increasing anti-Semitism in Nazi Germany, Auerbach immigrated to Britain, where she conducted research on the development of mutants in Drosophila. There she met Herman Muller, who had shown that radiation induces mutations; he suggested that Auerbach try to obtain mutants by treating Drosophila with chemicals. Her initial attempts met with little success. Other scientists were conducting top-secret research on mustard gas (used as a chemical weapon in World War I) and noticed that it produced many of the same effects as radiation. Auerbach was asked to determine whether mustard gas was mutagenic.

Collaborating with pharmacologist J. M. Robson, Auerbach studied the effects of mustard gas on Drosophila melanogaster. The experimental conditions were crude. They heated liquid mustard gas over a Bunsen burner on the roof of the pharmacology building, and the flies were exposed to the gas in a large chamber. After developing serious burns on her hands from the gas, Auerbach let others carry out the exposures, and she analyzed the flies. Auerbach and Robson showed that mustard gas is indeed a powerful mutagen, reducing the viability of gametes and increasing the numbers of mutations seen in the offspring of exposed flies. Because the research was part of the secret war effort, publication of their findings was delayed until 1947.

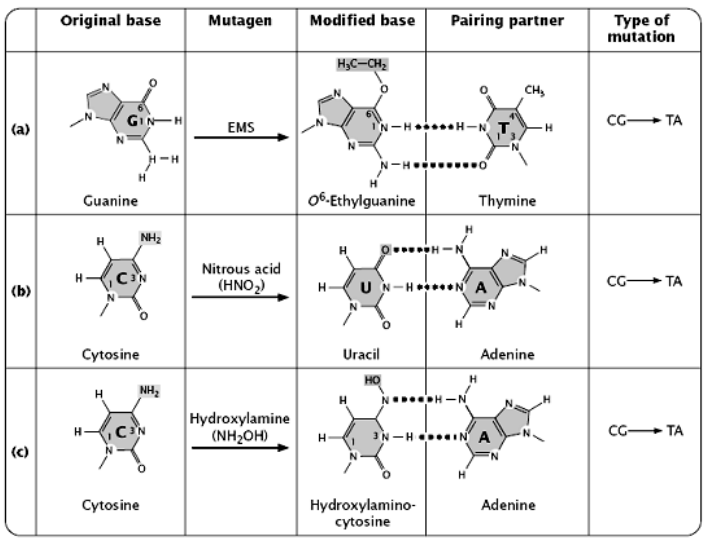
Base analogs One class of chemical mutagens consists of base analogs, chemicals with structures similar to that of any of the four standard bases of DNA. DNA polymerases cannot distinguish these analogs from the standard bases; so, if base analogs are present during replication, they may be incorporated into newly synthesized DNA molecules. For example, 5-bromouracil (5BU) is an analog of thymine; it has the same structure as that of thymine except that it has a bromine (Br) atom on the 5-carbon atom instead of a methyl group (FIGURE 16a). Normally, 5-bromouracil pairs with adenine just as thymine does, but it occasionally mispairs with guanine (FIGURE 16b), leading to a transition (T.A→5BU.A→5BU.G→C.G), as shown in FIGURE 17. Through mispairing, 5-bromouracil may also be incorporated into a newly synthesized DNA strand opposite guanine. In the next round of replication, 5-bromouracil may pair with adenine, leading to another transition (G.C→G.5BU→A.5BU→A.T).



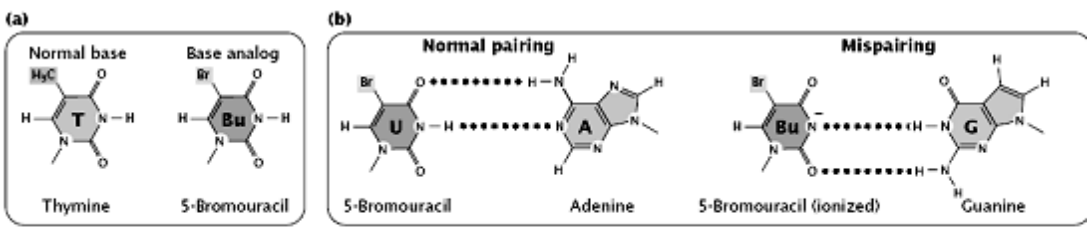
17. **5-Bromouracil can lead to a replicated error.**

Another mutagenic chemical is 2-aminopurine (2AP), which is a base analog of adenine (FIGURE 18). Normally, 2-aminopurine base pairs with thymine, but it may mispair with cytosine, causing a transition mutation (T.A→T.2AP→C.2AP→C.G). Alternatively, 2-aminopurine may be incorporated through mispairing into the newly synthesized DNA opposite cytosine and later pair with thymine, leading to a C.G→C.2AP→T.2AP→T.A transition.

Thus, both 5-bromouracil and 2-aminopurine can produce transition mutations. In the laboratory, mutations by base



18 Chemicals may alter DNA bases. (a) The alkylating agent ethylmethanesulfonate (EMS) adds an ethyl group to guanine, producing 6-ethylguanine, which pairs with thymine, producing a C·G→T·A transition mutation. (b) Nitrous acid deaminates cytosine to produce uracil, which pairs with adenine, producing a C·G→T·A transition mutation. (c) Hydroxylamine converts cytosine into hydroxylaminocytosine, which frequently pairs with adenine, leading to a C·G→T·A transition mutation.



16 5-Bromouracil (a base analog) resembles thymine, except that it has a bromine atom in place of a methyl group on the 5-carbon atom.

analogs can be reversed by treatment with the same analog or by treatment with a different analog. Alkylating agents Alkylating agents are chemicals that donate alkyl groups. These agents include methyl (CH_3) and ethyl (CH_3–CH_2) groups, which are added to nucleotide bases by some chemicals. For example, ethylmethanesulfonate (EMS) adds an ethyl group to guanine, producing 6-ethylguanine, which pairs with thymine. Thus, EMS produces C.G→T.A transitions. EMS is also capable of adding an ethyl group to thymine, producing 4-ethylthymine, which then pairs with guanine, leading to a T.A→C.G transition. Because EMS produces both C.G→T.A and T.A→C.G transitions,mutations produced by EMS can be reversed by additional treatment with EMS. Mustard gas is another alkylating agent.

Deamination

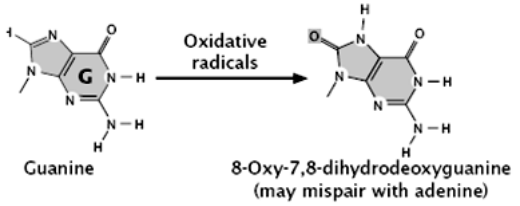
In addition to its spontaneous occurrence (see Figure 15), deamination can be induced by some chemicals. For instance, nitrous acid deaminates cytosine, creating uracil, which in the next round of replication pairs with adenine (see Figure 18b), producing a C.G→T.A transition mutation. Nitrous acid changes adenine into hypoxanthine, which pairs with cytosine, leading to a T.A→C.G transition. Nitrous acid also deaminates guanine, producing xanthine, which pairs with cytosine just as guanine does; however xanthine may also pair with thymine, leading to a C.G→T.A transition. Nitrous acid produces exclusively transition mutations and, because both C.G→T.A and T.A→C.G transitions are produced, these mutations can be reversed with nitrous acid.

Hydroxylamine

Hydroxylamine is a very specific basemodifying mutagen that adds a hydroxyl group to cytosine, converting it into hydroxylaminocytosine (see Figure 18c). This conversion increases the frequency of a rare tautomer that pairs with adenine instead of guanine and leads to C.G→T.A transitions. Because hydroxylamine acts only on cytosine, it will not generate T.A→C.G transitions; thus, hydroxylamine will not reverse the mutations that it produces.

Oxidative reactions

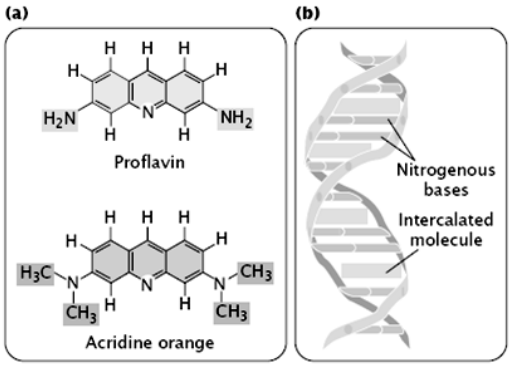
Reactive forms of oxygen (including superoxide radicals, hydrogen peroxide, and hydroxyl radicals) are produced in the course of normal aerobic metabolism, as well as by radiation, ozone, peroxides, and certain drugs. These reactive forms of oxygen damage DNA and induce mutations by bringing about chemical changes to DNA. For example, oxidation converts guanine into 8-oxy-7,8-dihydrodeoxyguanine (FIGURE 19), which frequently mispairs with adenine instead of cytosine, causing a G.C→T.A transversion mutation.



19 Oxidative radicals convert guanine into 8-oxy-7,8-dihydrodeoxyguanine, which frequently mispairs with adenine instead of cytosine, producing a G.C→T.A transversion

Intercalating agents Intercalating agents, such as proflavin, acridine orange, ethidium bromide, and dioxin are about the same size as a nucleotide (FIGURE 20a).

They produce mutations by sandwiching themselves (intercalating) between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single- nucleotide insertions and deletions in replication (FIGURE 20b). These insertions and deletions frequently produce frameshift mutations (which change all amino acids downstream of the mutation), and so the mutagenic effects of intercalating agents are often severe. Because intercalating agents generate both additions and deletions, they can reverse the effects of their own mutations.

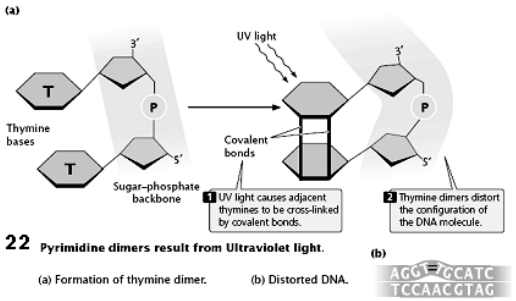


20 Intercalating agents such as proflavin and acridine orange insert themselves between adjacent bases in DNA, distorting the three-dimensional structure of the helix and causing single-nucleotide insertions and deletions in replication.

Radiation

In 1927, Herman Muller demonstrated that mutations in fruit flies could be induced by X-rays. The results of subsequent studies showed that X-rays greatly increase mutation rates in all organisms. The high energies of X-rays, gamma rays, and cosmic rays are all capable of penetrating tissues and damaging DNA. These forms of radiation, called ionizing radiation, dislodge electrons from the atoms that they encounter, changing stable molecules into free radicals and reactive ions, which then alter the structures of bases and break phosphodiester bonds in DNA. Ionizing radiation also frequently results in double-strand breaks in DNA. Attempts to repair these breaks can produce chromosome mutations.

Ultraviolet light has less energy than that of ionizing radiation and does not eject electrons and cause ionization but is nevertheless highly mutagenic. Purine and pyrimidine bases readily absorb UV light, resulting in the formation of chemical bonds between adjacent pyrimidine molecules on the same strand of DNA and in the creation of structures called pyrimidine dimers(FIGURE 22a). Pyrimidine dimers consisting of two thymine bases (called thymine dimers) are most frequent, but cytosine dimmers and thymine–cytosine dimers also can form. These dimmers distort the configuration of DNA (FIGURE 22b) and often block replication. Most pyrimidine dimers are immediately repaired by mechanisms, but some escape repair and inhibit replication and transcription.



22 **Pyrimidine dimers result from Ultraviolet light.**

(a) Formation of thymine dimer. (b) Distorted DNA.

When pyrimidine dimers block replication, cell division is inhibited and the cell usually dies; for this reason, UV light kills bacteria and is an effective sterilizing agent. For a mutation—a hereditary error in the genetic instructions—to occur, the replication block must be overcome.

How do bacteria and other organisms replicate despite the presence of thymine dimers? Bacteria can circumvent replication blocks produced by pyrimidine dimers and other types of DNA damage by means of the SOS system. This system allows replication blocks to be overcome, but in the process makes numerous mistakes and greatly increases the rate of mutation.

Indeed, the very reason that replication can proceed in the presence of a block is that the enzymes in the SOS system do not strictly adhere to the base-pairing rules. The trade-off is that replication may continue and the cell survives, but only by sacrificing the normal accuracy of DNA synthesis.

The SOS system is complex, including the products of at least 25 genes. A protein called RecA binds to the damaged DNA at the blocked replication fork and becomes activated. This activation promotes the binding of a protein called LexA, which is a repressor of the SOS system. The activated RecA complex induces LexA to undergo self-cleavage, destroying its repressive activity. This inactivation enables other SOS genes to be expressed, and the products of these genes allow replication of the damaged DNA to proceed. The SOS system allows bases to be inserted into a new DNA strand in the absence of bases on the template strand, but these insertions result in numerous errors in the base sequence.

Eukaryotic cells have a specialized DNA polymerase called polymerase η (eta) that bypasses pyrimidine dimers. Polymerase η preferentially inserts AA opposite a pyrimidine dimer. This strategy seems to be reasonable because about two-thirds of pyrimidine dimers are thymine dimers. However, the insertion of AA opposite a CT dimer results in a C.G→A.T transversion. Polymerase η is therefore said to be an error-prone polymerase.

The Study of Mutations

Because mutations often have detrimental effects, they have been the subject of intense study by geneticists. These studies have included the analysis of reverse mutations, which are often sources of important insight into how mutations cause DNA damage; the development of tests to determine the mutagenic properties of chemical compounds; and the investigation of human populations tragically exposed to high levels of radiation.

The Analysis of Reverse Mutations

The study of reverse mutations (reversions) can provide useful information about how mutagens alter DNA structure. For example, any mutagen that produces both A.T→G.C and G.C:A.T transitions should be able to reverse its own mutations. However, if the mutagen produces only G.C→A.T transitions, then reversion by the same mutagen is not possible. Hydroxylamine (see Figure 18c) exhibits this type of one-way mutagenic activity; it causes G.C→A.T transitions but is incapable of reversing the mutations that it produces; so we know that it does not produce A.T→G.C transitions. Ethylmethanesulfonate (see Figure 18a), on the other hand, produces G.C→A.T transitions and reverses its own mutations; so we know that it also produces T.A→C.G transitions.

Analyses of the ability of different mutagens to cause reverse mutations can be sources of insight into the molecular nature of the mutations. We can use reverse mutations to determine whether a mutation results from a base substitution or a frameshift. Base analogs such as 2-aminopurine cause transitions, and intercalating agents such as acridine orange (see Figure 20) produce frameshifts. If a chemical reverses mutations produced by 2-aminopurine but not those produced by acridine orange, we can conclude that the chemical causes transitions and not frameshifts. If nitrous acid (which produces both G.C→A.T and A.T→G.C transitions) reverses mutations produced by the chemical but hydroxylamine (which causes only G.C→A.T transitions) does not, we know that, like hydroxylamine, the chemical produces only G.C→A.T transitions. Table 4 illustrates the reverse mutations that are theoretically possible among several mutagenic agents. The actual ability of mutagens to produce reversals is more complex than suggested by Table 4 and depends on environmental conditions and the organism tested.

Table 4 Theoretical reverse mutations possible by various mutagenic agents

		Reversal of Mutations by					
Mutagen	**Type of Mutation**	**5-Bromo-uracil**	**2-Amino-purine**	**Ethyl methane sulfonate**	**Nitrous acid**	**Hydroxyl-amine**	**Acridine orange**
5-Bromouracil	C·G↔T·A	+	+	+	+	+/−	−
2-Aminopurine	C·G↔T·A	+	+	+	+	+/−	−
Nitrous acid	C·G↔T·A	+	+	+	+	+/−	−
Ethylmethane sulfonate	C·G↔T·A	+	+	+	+	+/−	−
Hydroxylamine	C·G↔T·A	+	+	+	+	−	−
Acridine orange	Frameshift	−	−	−	−	−	+

Note: + indicates that reverse mutations occur, − indicates that reverse mutations do not occur, and +/− indicates that only some mutations are reversed. Not all reverse mutations are equally likely.

Detecting Mutations with the Ames Test

Humans in industrial societies are surrounded by a multitude of artificially produced chemicals: more than 50,000 different chemicals are in commercial and industrial use today, and from 500 to 1000 new chemicals are

introduced each year. Some of these chemicals are potential carcinogens and may cause potential harm to humans. How can we determine which chemicals are hazardous? In a few instances, previous human exposure to a specific chemical is correlated with an increase in cancer incidence, providing good evidence that the chemical is a carcinogen. But, ideally, we would like to know which chemicals are hazardous before we are exposed to them. One method for testing the cancer-causing potential of chemicals is to administer them to laboratory animals (rats or mice) and compare the incidence of cancer in the treated animals with that of control animals. These tests are unfortunately time consuming and expensive. Furthermore, the ability of a substance to cause cancer in rodents is not always indicative of its effect on humans. After all, we aren't rats!

In 1974, Bruce Ames developed a simple test for evaluating the potential of chemicals to cause cancer. The Ames test is based on the principle that both cancer and mutations result from damage to DNA, and the results of experiments have demonstrated that 90% of known carcinogens are also mutagens. Ames proposed that mutagenesis in bacteria could serve as an indicator of carcinogenesis in humans. The Ames test uses four strains of the bacterium *Salmonella typhimurium* that have defects in the lipopolysaccharide coat, which normally protects the bacteria from chemicals in the environment. Furthermore, their DNA repair system has been inactivated, enhancing their susceptibility to mutagens.

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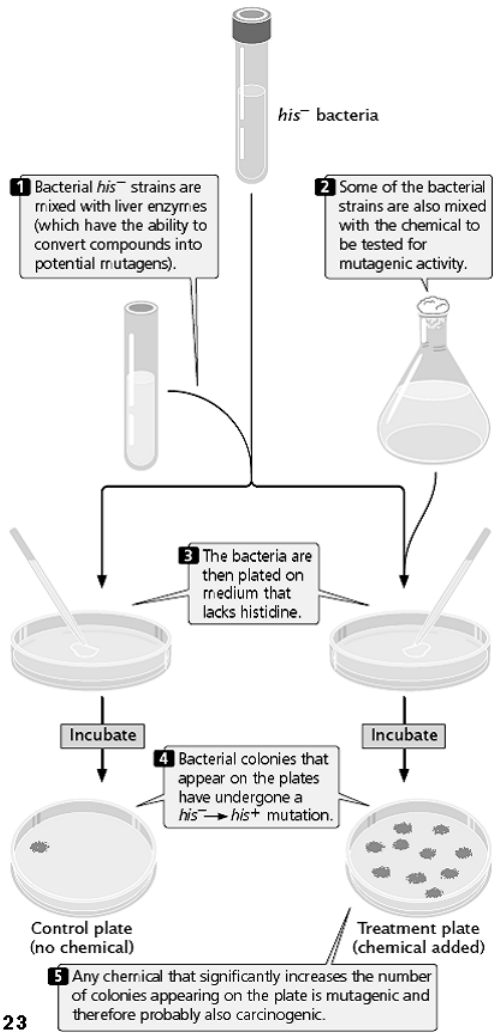
One of the four strains used in the Ames test detects base-pair substitutions; the other three detect different types of frameshift mutations. Each strain carries a mutation that renders it unable to synthesize the amino acid histidine (his^-), and the bacteria are plated onto medium that lacks histidine (FIGURE 23). Only bacteria that have undergone a reverse mutation of the histidine gene ($his^- \rightarrow his^+$) are able to synthesize histidine and grow on the medium. Different dilutions of a chemical to be tested are added to plates inoculated with the bacteria, and the number of mutant bacterial colonies that appear on each plate is compared with the number that appear on control plates with no chemical (arose through spontaneous mutation). Any chemical that significantly increases the number of colonies appearing on a treated plate is mutagenic and is probably also carcinogenic.

Some compounds are not active carcinogens but may be converted into cancer-causing compounds in the body. To make the Ames test sensitive for such potential carcinogens, a compound to be tested is first incubated in mammalian liver extract that contains metabolic enzymes. The Ames test has been applied to thousands of chemicals and commercial products. An early demonstration of its usefulness was the discovery, in 1975, that most hair dyes sold in the United States contained compounds that were mutagenic to bacteria. These compounds were then removed from most hair dyes.



23

The Ames test is used to identify chemical mutagens.

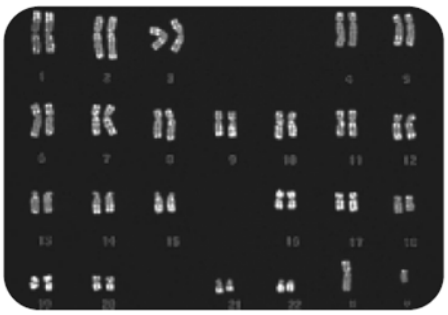
J. Structural and numerical alterations of chromosomes: Deletion, duplication, inversion, translocation, ploidy and their genetic implications.

Chromosome Variation

Before we consider the different types of chromosome mutations, their effects, and how they arise, we will review the basics of chromosome structure.

Chromosome Morphology

Each functional chromosome has a centromere, where spindle fibers attach, and two telomeres that stabilize the chromosome. Chromosomes are classified into four basic types: metacentric, in which the centromere is located approximately in the middle, and so the chromosome has two arms of equal length; sub-metacentric, in which the centromere is displaced toward one end, creating a long arm and a short arm; acrocentric, in which the centromere is near one end, producing a long arm and a knob, or satellite, at the other; and telocentric, in which the centromere is at or very near the end of the chromosome. On human chromosomes, the short arm is designated by the letter p and the long arm by the letter q.

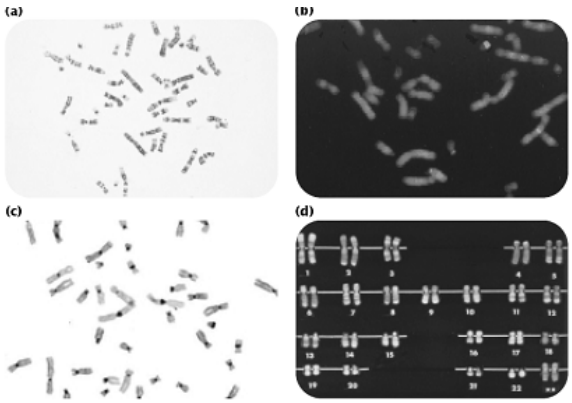


1 A human karyotype consists of 46 chromosomes. A karyotype for a male is shown here; a karyotype for a female would have two X chromosomes.

The complete set of chromosomes that an organism possesses is called its karyotype and is usually presented as a picture of metaphase chromosomes lined up in descending order of their size (FIGURE 1). Karyotypes are prepared from actively dividing cells, such as white blood cells, bone marrow cells, or cells from meristematic tissues of plants.

After treatment with a chemical (such as colchicine) that prevents them from entering anaphase, the cells are chemically preserved, spread on a microscope slide, stained, and photographed. The photograph is then enlarged, and the individual chromosomes are cut out and arranged in a karyotype. For human chromosomes, karyotypes are often routinely prepared by automated machines, which scan a slide with a video camera attached to a microscope, looking for chromosome spreads. When a spread has been located, the camera takes a picture of the chromosomes, the image is digitized, and the chromosomes are sorted and arranged electronically by a computer.

Preparation and staining techniques have been developed to help distinguish among chromosomes of similar size and shape. For instance, chromosomes may be treated with enzymes that partly digest them; staining with a special dye called Giemsa reveals G bands, which distinguish areas of DNA that are rich in adenine–thymine base pairs (FIGURE 2a). Q bands (FIGURE 2b) are revealed by staining chromosomes with quinacrine mustard and viewing the chromosomes under UV light. Other techniques reveal C bands (FIGURE 2c), which are regions of DNA occupied by centromeric heterochromatin, and R bands (FIGURE 2d), which are rich in guanine – cytosine base pairs.



2 Chromosome banding is revealed by special staining techniques.

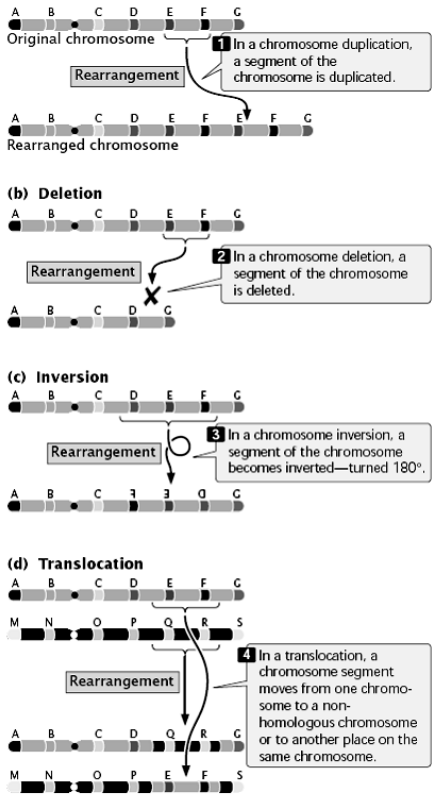
Types of Chromosome Mutations

Chromosome mutations can be grouped into three basic categories: chromosome rearrangements, aneuploids, and polyploids.

Chromosome rearrangements alter the structure of chromosomes; for example, a piece of a chromosome might be duplicated, deleted, or inverted. In aneuploidy, the number of chromosomes is altered: one or more individual chromosomes are added or deleted. In polyploidy, one or more complete sets of chromosomes are added. Some organisms (such as yeast) possess a single chromosome set (1n) for most of their life cycles and are referred to as haploid, whereas others possess two chromosome sets and are referred to as diploid (2n). A polyploid is any organism that has more than two sets of chromosomes (3n, 4n, 5n, or more).

Chromosome Rearrangements

Chromosome rearrangements are mutations that change the structure of individual chromosomes. The four basic types of rearrangements are duplications, deletions, inversions, and translocations (FIGURE 3).



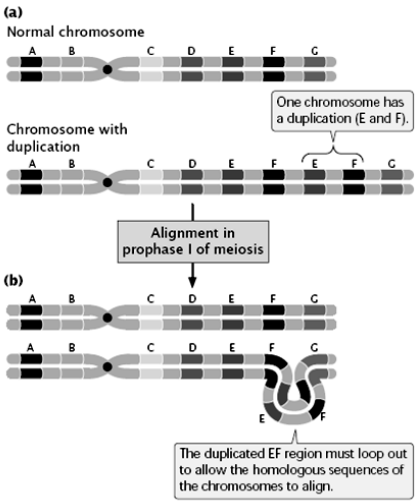
3 The four basic types of chromosome rearrangements are duplication, deletion, inversion, and translocation.

Duplications

A chromosome duplication is a mutation in which part of the chromosome has been doubled (see Figure 3a). Consider a chromosome with segments AB•CDEFG, in which • represents the centromere. A duplication might include the EF segments, giving rise to a chromosome with segments AB•CDEFEFG. This type of duplication, in which the duplicated region is immediately adjacent to the original segment, is called a tandem duplication. If the duplicated segment is located some distance from the original segment, either on the same chromosome or on a different one, this type is called a displaced duplication. An example of a displaced duplication would be AB•CDEFGEF. A duplication can either be in the same orientation as the original sequence, as in the two preceding examples, or be inverted: AB•CDEFEFG. When the duplication is inverted, it is called a reverse duplication.

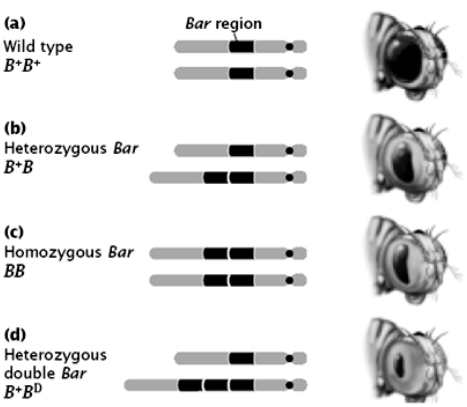
An individual homozygous for a rearrangement carries the rearrangement (the mutated sequence) on both homologous chromosomes, and an individual heterozygous for a rearrangement has one unmutated chromosome and one chromosome with the rearrangement. In the heterozygotes (FIGURE 4a), problems arise in chromosome pairing at prophase I of meiosis, because the two chromosomes are not homologous throughout their length. The homologous

regions will pair and undergo synapsis, which often requires that one or both chromosomes loop and twist so that these regions are able to line up (FIGURE 4b). The appearance of this characteristic loop structure during meiosis is one way to detect duplications.



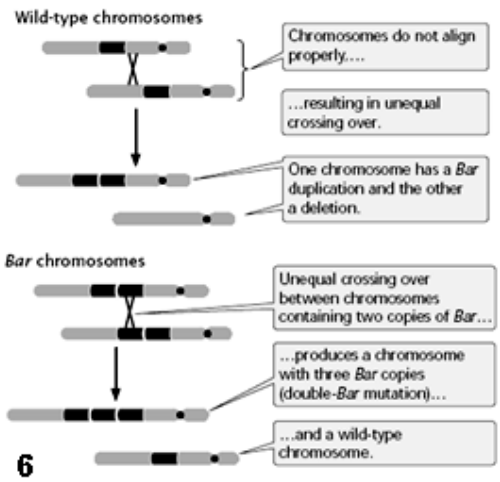
4 In an individual heterozygous for a duplication, the duplicated chromosome loops out during pairing in prophase I.

Duplications may have major effects on the phenotype. In Drosophila melanogaster, for example, a Bar mutant has a reduced number of facets in the eye, making the eye smaller and bar shaped instead of oval (FIGURE 5). The Bar mutant results from a small duplication on the X chromosome, which is inherited as an incompletely dominant, X-linked trait: heterozygous female flies have somewhat smaller eyes (the number of facets is reduced; see Figure 5), whereas, in homozygous female and hemizygous male flies, the number of facets is greatly reduced (see Figure 5c). Occasionally, a fly carries three copies of the Bar duplication on its X chromosome; in such mutants, which are termed double Bar, the number of facets is extremely reduced (see Figure 5d). Bar arises from unequal crossing over, a duplication-generating process (FIGURE 6).



5 The Bar phenotype in *Drosophila melanogaster* results from an X-linked duplication. (a) Wild-type fruit flies have normal-size eyes. (b) Flies heterozygous and (c) homozygous for the *Bar* mutation have smaller, bar-shaped eyes. (d) Flies with double *Bar* have three copies of the duplication and much smaller bar-shaped eyes.

How does a chromosome duplication alter the phenotype? After all, gene sequences are not altered by duplications, and no genetic information is missing; the only change is the presence of additional copies of normal sequences. The answer to this question is not well understood, but the effects are most likely due to imbalances in the amounts of gene products (abnormal gene dosage). The amount of a particular protein synthesized by a cell is often directly related to the number of copies of its corresponding gene: an individual with three functional copies of a gene often produces 1.5 times as much of the protein encoded by that gene as that produced by an individual with two copies.



Unequal crossing over produces *Bar* and double-*Bar* mutations.

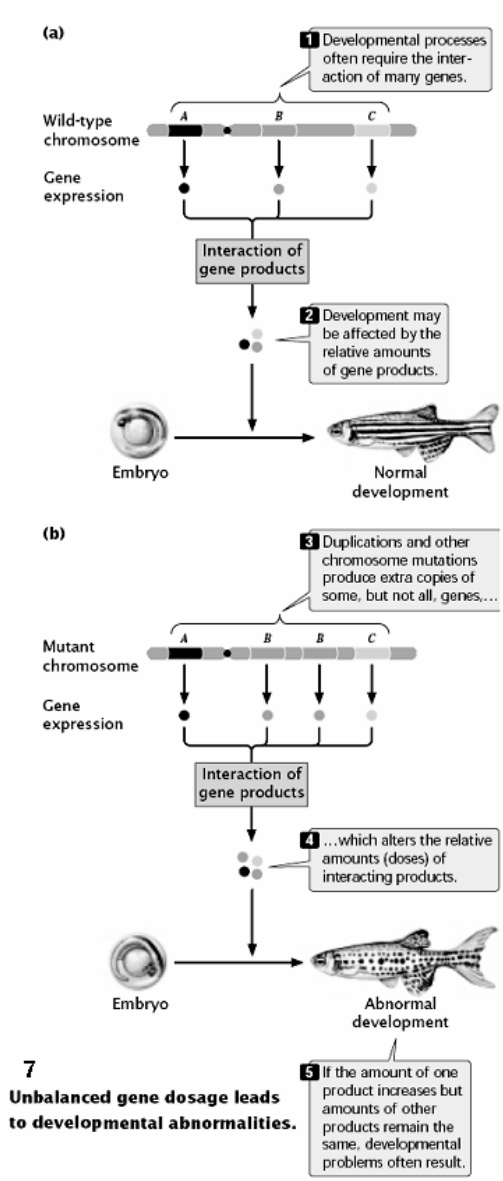
Because developmental processes often require the interaction of many proteins, they may critically depend on the relative amounts of the proteins. If the amount of one protein increases while the amounts of others remain constant, problems can result (FIGURE 7).

Although duplications can have severe consequences when the precise balance of a gene product is critical to cell function, duplications have arisen frequently throughout the evolution of many eukaryotic organisms and are a source of new genes that may provide novel functions. Human phenotypes associated with some duplications are summarized in Table 1.

Deletions

A second type of chromosome rearrangement is a deletion, the loss of a chromosome segment (see Figure 3b). A chromosome with segments AB•CDEFG that undergoes a deletion of segment EF would generate the mutated chromosome AB•CDG.

A large deletion can be easily detected because the chromosome is noticeably shortened. In individuals heterozygous for deletions, the normal chromosome must loop out during the pairing of homologs in prophase I of meiosis to allow the homologous regions of the two chromosomes to align and undergo synapsis. This looping out generates a structure that looks very much like that seen in individuals heterozygous for duplications.



Unbalanced gene dosage leads to developmental abnormalities.

The phenotypic consequences of a deletion depend on which genes are located in the deleted region. If the deletion includes the centromere, the chromosome will not segregate in meiosis or mitosis and will usually be lost. Many deletions are lethal in the homozygous state because all copies of any essential genes located in the deleted region are missing. Even individuals heterozygous for a deletion may have multiple defects for three reasons. First, the heterozygous condition may produce imbalances in the amounts of gene products, similar to the imbalances produced by extra gene copies. Second, deletions may allow recessive mutations on the undeleted chromosome to be expressed (because there is no wild-type allele to mask their expression).

Table 1 Effects of some chromosome rearrangements in humans

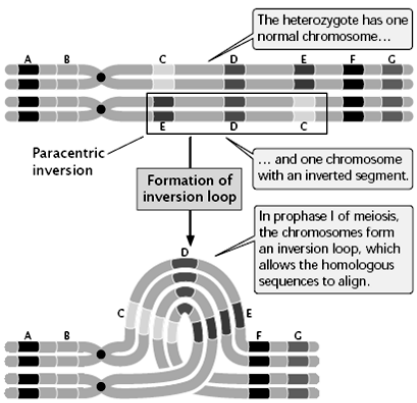
Type of Rearrangement	Chromosome	Disorder	Symptoms
Duplication	4, short arm	—	Small head, short neck, low hairline, growth and mental retardation
Duplication	4, long arm	—	Small head, sloping forehead, hand abnormalities
Duplication	7, long arm	—	Delayed development, asymmetry of the head, fuzzy scalp, small nose, low-set ears
Duplication	9, short arm	—	Characteristic face, variable mental retardation, high and broad forehead, hand abnormalities
Deletion	5, short arm	*Cri-du-chat* syndrome	Small head, distinctive cry, widely syndrome spaced eyes, a round face, mental retardation
Deletion	4, short arm	Wolf-Hirschhorn syndrome	Small head with high forehead, wide nose, cleft lip and palate, severe mental retardation
Deletion	4, long arm	—	Small head, mild to moderate mental retardation, cleft lip and palate, hand and foot abnormalities
Deletion	15, long arm	Prader-Willi syndrome	Feeding difficulty at early age, but becoming obese after 1 year of age, mild to moderate mental retardation
Deletion	18, short arm	—	Round face, large low set-ears, mild to moderate mental retardation
Deletion	18, long arm	—	Distinctive mouth shape, small hands, small head, mental retardation

This phenomenon is referred to as pseudodominance. The appearance of pseudodominance in otherwise recessive alleles is an indication that a deletion is present on one of the chromosomes. Third, some genes must be present in two copies for normal function. Such a gene is said to be haploinsufficient; loss of function mutations in haploinsufficient genes are dominant. Notch is a series of X-linked wing mutations in Drosophila that often result from chromosome deletions. Notch deletions behave as dominant mutations: when heterozygous for the Notch deletion, a fly has wings that are notched at the tips and along the edges. The Notch locus is therefore haploinsufficient—a single copy of the gene is not sufficient to produce a wild-type phenotype. Females that are homozygous for a Notch deletion (or males that are hemizygous) die early in embryonic development. The Notch gene codes for a receptor that normally transmits signals received from outside the cell to the cell's interior and is important in fly development. The deletion acts as a recessive lethal because loss of all copies of the Notch gene prevents normal development.

In humans, a deletion on the short arm of chromosome 5 is responsible for cri-du-chat syndrome. The name (French for "cry of the cat") derives from the peculiar, catlike cry of infants with this syndrome. A child who is heterozygous for this deletion has a small head, widely spaced eyes, a round face, and mental retardation. Deletion of part of the short arm of chromosome 4 results in another human disorder—Wolf-Hirschhorn syndrome, which is characterized by seizures and by severe mental and growth retardation.

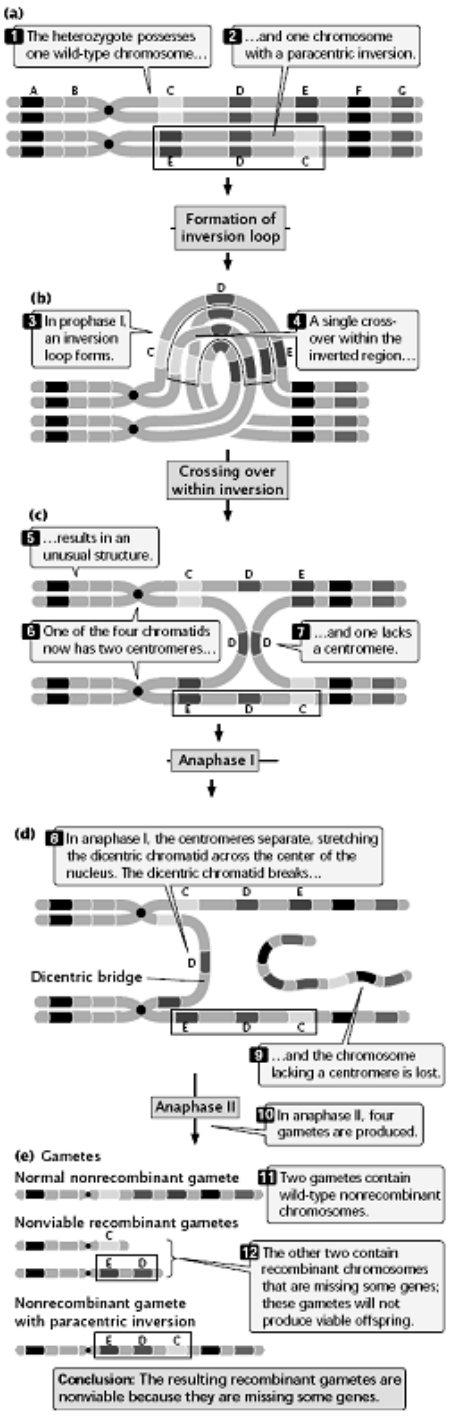
A third type of chromosome rearrangement is a chromosome inversion, in which a chromosome segment is inverted—turned 180 degrees (see Figure 9.5c). If a chromosome originally had segments AB•CDEFG, then chromosome AB•CFEDG represents an inversion that includes segments DEF. For an inversion to take place, the chromosome must break in two places. Inversions that do not include the centromere, such as AB•CFEDG, are termed paracentric inversions (para meaning "next to"), whereas inversions that include the centromere, such as ADC•BEFG, are termed pericentric inversions (peri meaning "around").

Individuals with inversions have neither lost nor gained any genetic material; just the gene order has been altered. Nevertheless, these mutations often have pronounced phenotypic effects. An inversion may break a gene into two parts, with one part moving to a new location and destroying the function of that gene. Even when the chromosome breaks are between genes, phenotypic effects may arise from the inverted gene order in an inversion. Many genes are regulated in a position-dependent manner; if their positions are altered by an inversion, they may be expressed at inappropriate times or in inappropriate tissues. This outcome is referred to as a position effect.



8 In an individual heterozygous for a paracentric inversion, the chromosomes form an inversion loop during pairing in prophase I.

When an individual is homozygous for a particular inversion, no special problems arise in meiosis, and the two homologous chromosomes can pair and separate normally. When an individual is heterozygous for an inversion, however, the gene order of the two homologs differs, and the homologous sequences can align and pair only if the two chromosomes form an inversion loop (FIGURE 8).



9. **In a heterozygous individual, a single crossover within a paracentric inversion leads to abnormal gametes.**

The presence of an inversion loop in meiosis indicates that an inversion is present. Individuals heterozygous for inversions also exhibit reduced recombination among genes located in the inverted region. The frequency of crossing over within the inversion is not actually diminished but, when crossing over does take place, the result is a tendency to produce gametes that are not viable and thus no recombinant progeny are observed.

Let's see why this occurs. FIGURE 9 illustrates the results of crossing over within a paracentric inversion. The individual is heterozygous for an inversion (see Figure 9a), with one wild-type, unmutated chromosome (AB•CDEFG) and one inverted chromosome (AB•EDCFG). In prophase I of meiosis, an inversion loop forms, allowing the homologous sequences to pair up (see Figure 9b). If a single crossover takes place in the inverted region (between segments C and D in Figure 9), an unusual structure results (see Figure 9c). The two outer chromatids, which did not participate in crossing over, contain original, nonrecombinant gene sequences. The two inner chromatids, which did cross over, are highly abnormal: each has two copies of some genes and no copies of others. Furthermore, one of the four chromatids now has two centromeres and is said to be dicentric; the other lacks a centromere and is acentric.

In anaphase I of meiosis, the centromeres are pulled toward opposite poles and the two homologous chromosomes separate. This stretches the dicentric chromatid across the center of the nucleus, forming a structure called a dicentric bridge (see Figure 9d). Eventually the dicentric bridge breaks, as the two centromeres are pulled farther apart. The acentric fragment has no centromere. Spindle fibers do not attach to it, and so this fragment does not segregate into a nucleus in meiosis and is usually lost. In the second division of meiosis, the chromatids separate and four gametes are produced (see Figure 9e). Two of the gametes contain the original, non-recombinant chromosomes (AB•CDEFG and AB•EDCFG). The other two gametes contain recombinant chromosomes that are missing some genes; these gametes will not produce viable offspring.

Thus, no recombinant progeny result when crossing over takes place within a paracentric inversion. Recombination is also reduced within a pericentric inversion (FIGURE 10). No dicentric bridges or acentric fragments are produced, but the recombinant chromosomes have too many copies of some genes and no copies of others; so gametes that receive the recombinant chromosomes cannot produce viable progeny. Figures 9 and 10 illustrate the results of single crossovers within inversions. Double crossovers, in which both crossovers are on the same two strands (two-strand, double crossovers), result in functional, recombinant chromosomes. Thus, even though the overall rate of recombination is reduced within an inversion, some viable recombinant progeny may still be produced through two-stranded double crossovers.

Inversion heterozygotes are common in many organisms, including a number of plants, some species of Drosophila, mosquitoes, and grasshoppers. Inversions may have played an important role in human evolution: G-banding patterns reveal that several human chromosomes differ from those of chimpanzees by only a pericentric inversion (FIGURE 11).

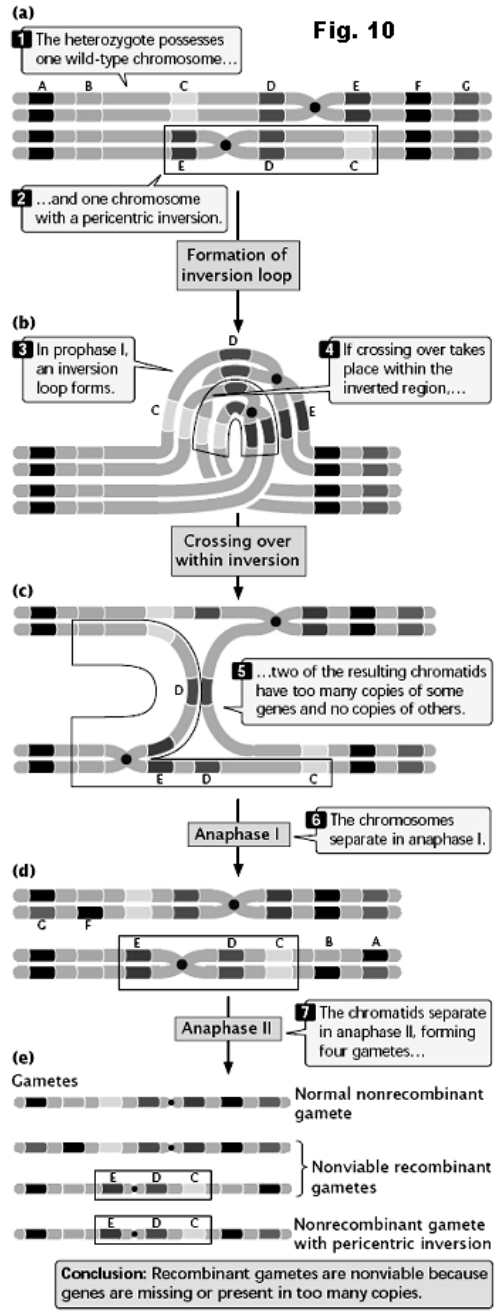
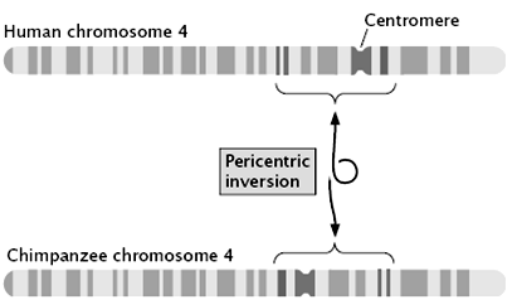


Fig. 10



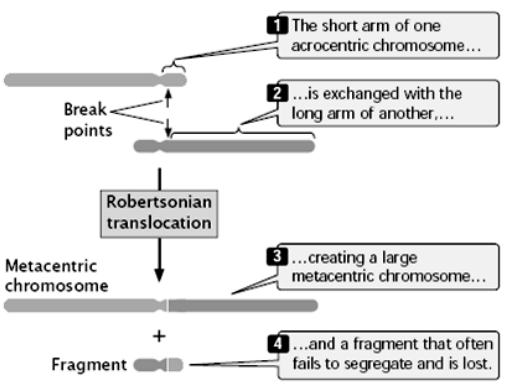
11 Chromosome 4 differs in humans and chimpanzees in a pericentric inversion.

Translocations

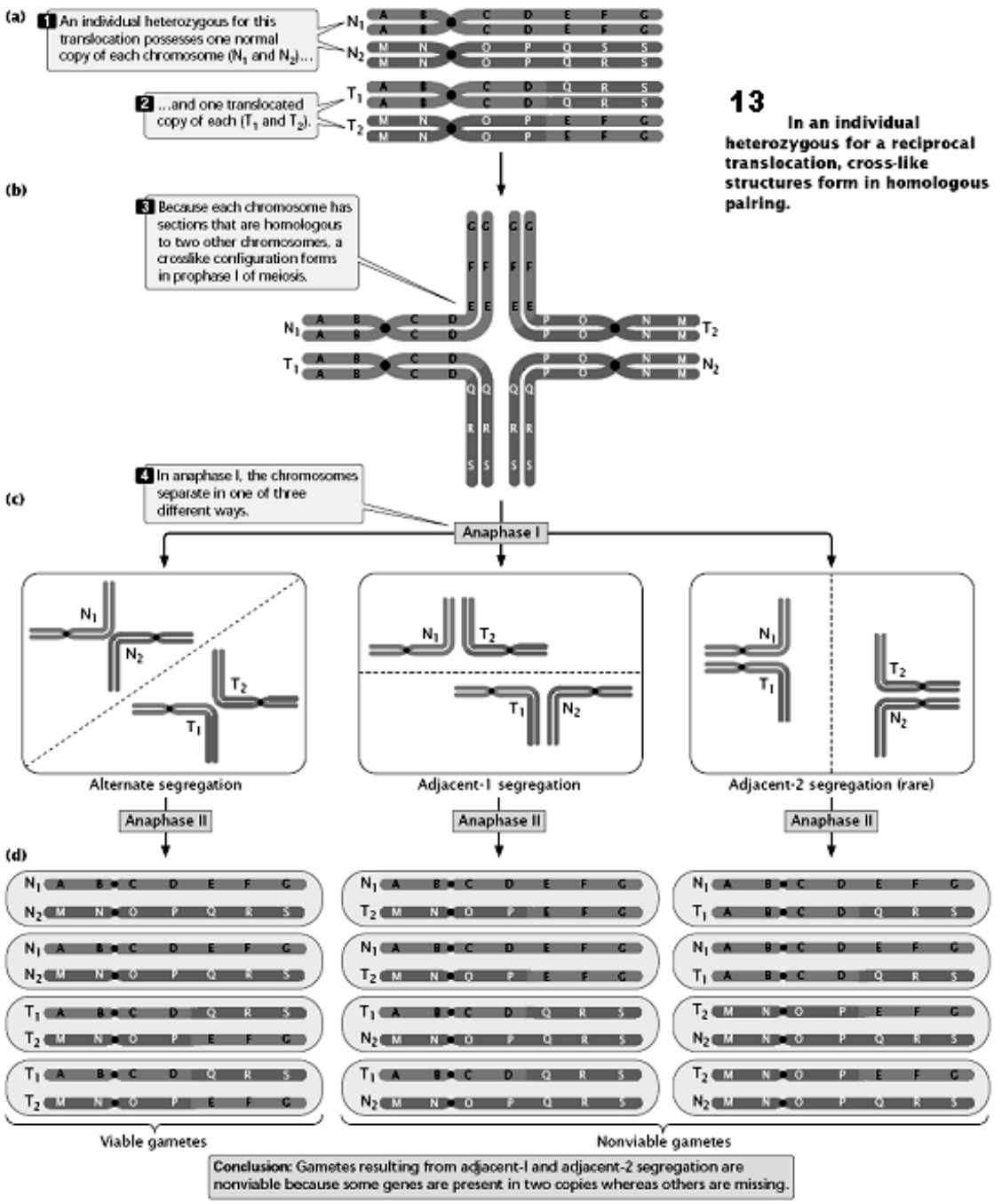
A translocation entails the movement of genetic material between non-homologous chromosomes (see Figure 3d) or within the same chromosome. Translocation should not be confused with crossing over, in which there is an exchange of genetic material between homologous chromosomes.

In nonreciprocal translocations, genetic material moves from one chromosome to another without any reciprocal exchange. Consider the following two non-homologous chromosomes: AB•CDEFG and MN•OPQRS. If chromosome segment EF moves from the first chromosome to the second without any transfer of segments from the second chromosome to the first, a nonreciprocal translocation has taken place, producing chromosomes AB•CDG and MN•OPEFQRS. More commonly, there is a two-way exchange of segments between the chromosomes, resulting in a reciprocal translocation. A reciprocal translocation between chromosomes AB•CDEFG and MN•OPQRS might give rise to chromosomes AB•CDQRG and MN•OPEFS. Translocations can affect a phenotype in several ways. First, they may create new linkage relations that affect gene expression (a position effect): genes translocated to new locations may come under the control of different regulatory sequences or other genes that affect their expression— an example is found in Burkitt lymphoma.

Second, the chromosomal breaks that bring about translocations may take place within a gene and disrupt its function. Molecular geneticists have used these types of effects to map human genes. Neurofibromatosis is a genetic disease characterized by numerous fibrous tumors of the skin and nervous tissue; it results from an autosomal dominant mutation. Linkage studies first placed the locus for neurofibromatosis on chromosome 17. Geneticists later identified two patients with neurofibromatosis who possessed a translocation affecting chromosome 17. These patients were assumed to have developed neurofibromatosis because one of the chromosome breaks that occurred in the translocation disrupted a particular gene that causes neurofibromatosis. DNA from the regions around the breaks was sequenced and eventually led to the identification of the gene responsible for neurofibromatosis.



12 In a Robertsonian translocation, the short arm of one acrocentric chromosome is exchanged with the long arm of another.



13

In an individual heterozygous for a reciprocal translocation, cross-like structures form in homologous pairing.

Deletions frequently accompany translocations. In a Robertsonian translocation, for example, the long arms of two acrocentric chromosomes become joined to a common centromere through a translocation, generating a metacentric chromosome with two long arms and another chromosome with two very short arms (FIGURE 12). The smaller chromosome often fails to segregate, leading to an overall reduction in chromosome number. As we will see, Robertsonian translocations are the cause of some cases of Down syndrome.

The effects of a translocation on chromosome segregation in meiosis depend on the nature of the translocation. Let us consider what happens in an individual heterozygous for a reciprocal translocation. Suppose that the original chromosome segments were AB•CDEFG and MN•OPQRS (designated N1 and N2), and a reciprocal translocation takes place, producing chromosomes AB•CDQRS and MN•OPEFG (designated T1 and T2). An individual heterozygous for this translocation would possess one normal copy of each chromosome and one translocated copy (FIGURE 13a). Each of these chromosomes contains segments that are homologous to two other chromosomes. When the homologous sequences pair in prophase I of meiosis, crosslike configurations consisting of all four chromosomes (FIGURE 13b) form. Notice that N_1 and T_1 have homologous centromeres (in both chromosomes the centromere is between segments B and C); similarly, N_2 and T_2 have homologous centromeres (between segments N and O). Normally, homologous centromeres separate and move

toward opposite poles in anaphase I of meiosis. With a reciprocal translocation, the chromosomes may segregate in three different ways. In alternate segregation (FIGURE 13c), N_1 and N_2 move toward one pole and T_1 and T_2 move toward the opposite pole. In adjacent-1 segregation, N_1 and T_2 move toward one pole and T_1 and N_2 move toward the other pole. In both alternate and adjacent-1 segregation, homologous centromeres segregate toward opposite poles. Adjacent-2 segregation, in which N_1 and T_1 move toward one pole and T_2 and N_2 move toward the other, is rare.

The products of the three segregation patterns are illustrated in FIGURE 13d. As you can see, the gametes produced by alternate segregation possess one complete set of the chromosome segments. These gametes are therefore functional and can produce viable progeny. In contrast, gametes produced by adjacent-1 and adjacent-2 segregation are not viable, because some chromosome segments are present in two copies, whereas others are missing. Adjacent-2 segregation is rare, and so most gametes are produced by alternate and adjacent segregation. Therefore, approximately half of the gametes from an individual heterozygous for a reciprocal translocation are expected to be functional.

Translocations can play an important role in the evolution of karyotypes. Chimpanzees, gorillas, and orangutans all have 48 chromosomes, whereas humans have 46. Human chromosome 2 is a large, metacentric chromosome with G-banding patterns that match those found on two different acrocentric chromosomes of the apes (FIGURE 14). Apparently, a Robertsonian translocation took place in a human ancestor, creating a large metacentric chromosome from the two long arms of the ancestral acrocentric chromosomes and a small chromosome consisting of the two short arms. The small chromosome was subsequently lost, leading to the reduced human chromosome number.

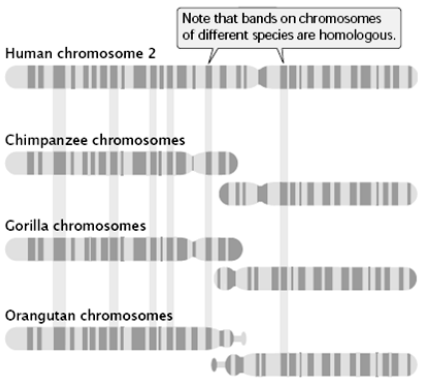
Fragile Sites

Chromosomes of cells grown in culture sometimes develop constrictions or gaps at particular locations called fragile sites (FIGURE 15) because they are prone to breakage under certain conditions. A number of fragile sites have been identified on human chromosomes. One of the most intensively studied is a fragile site on the human X chromosome that is associated with mental retardation, the fragile-X syndrome. Exhibiting X-linked inheritance and arising with a frequency of about 1 in 1250 male births, fragile-X syndrome has been shown to result from an increase in the number of repeats of a CGG trinucleotide. However, other common fragile sites do not consist of trinucleotide repeats, and their nature is still incompletely understood.

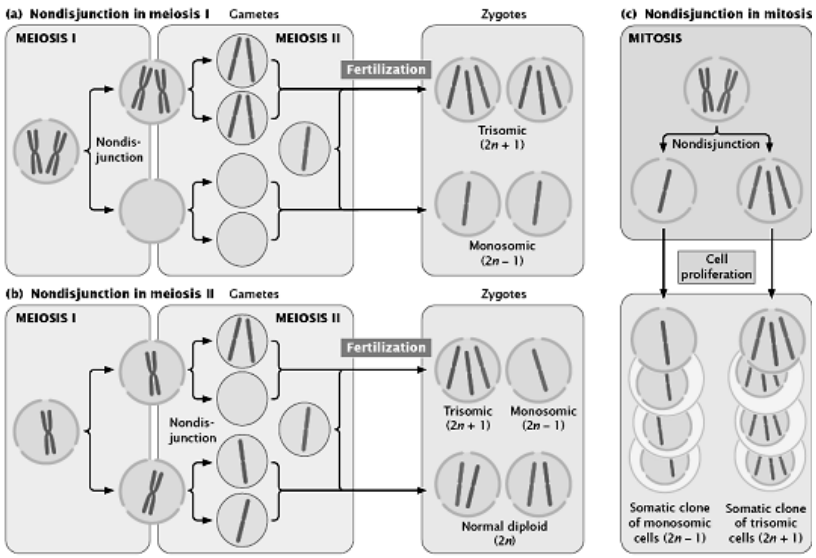
Aneuploidy

In addition to chromosome rearrangements, chromosome mutations also include changes in the number of chromosomes. Variations in chromosome number can be classified into two basic types: changes in the number of individual chromosomes (aneuploidy) and changes in the number of chromosome sets (polyploidy).

Aneuploidy can arise in several ways. First, a chromosome may be lost in the course of mitosis or meiosis if, for example, its centromere is deleted. Loss of the centromere prevents the spindle fibers from attaching; so the chromosome fails to move to the spindle pole and does not become incorporated into a nucleus after cell division. Second, the small chromosome generated by a Robertsonian translocation may be lost in mitosis or meiosis. Third, aneuploids may arise through nondisjunction, the failure of homologous chromosomes or sister chromatids to separate in meiosis or mitosis. Nondisjunction leads to some gametes or cells that contain an extra chromosome and others that are missing a chromosome (FIGURE 15).



14 Human chromosome 2 contains a Robertsonian translocation that is not present in chimps, gorillas, or orangutans. G-banding reveals that a Robertsonian translocation in a human ancestor switched the long and short arms of the two acrocentric chromosomes that are still found in the other three primates. This translocation created the large metacentric human chromosome 2.



15 Aneuploids can be produced through nondisjunction in (a) meiosis I, (b) meiosis II, and (c) mitosis. The gametes that result from meioses with nondisjunction combine with gamete (with blue chromosome) that results from normal meiosis to produce the zygotes.

Types of Aneuploidy

We will consider four types of relatively common aneuploid conditions in diploid individuals: nullisomy, monosomy, trisomy, and tetrasomy.

1. Nullisomy is the loss of both members of a homologous pair of chromosomes. It is represented as 2n - 2, where n refers to the haploid number of chromosomes. Thus, among humans, who normally possess 2n = 46 chromosomes, a nullisomic person has 44 chromosomes.

2. Monosomy is the loss of a single chromosome, represented as 2n - 1. A monosomic person has 45 chromosomes.

3. Trisomy is the gain of a single chromosome, represented as 2n + 1. A trisomic person has 47 chromosomes. The gain of a chromosome means that there are three homologous copies of one chromosome.

4. Tetrasomy is the gain of two homologous chromosomes, represented as 2n + 2. A tetrasomic person has 48 chromosomes. Tetrasomy is not the gain of any two extra chromosomes, but rather the gain of two homologous chromosomes; so there will be four homologous copies of a particular chromosome.

More than one aneuploid mutation may occur in the same individual. An individual that has an extra copy of two different (non-homologous) chromosomes is referred to as being double trisomic and represented as 2n + 1 + 1. Similarly, a double monosomic has two fewer non-homologous chromosomes (2n - 1 - 1), and a double tetrasomic has two extra pairs of homologous chromosomes (2n + 2 + 2).

Effects of Aneuploidy

One of the first aneuploids to be recognized was a fruit fly with a single X chromosome and no Y chromosome, which was discovered by Calvin Bridges in 1913. Another early study of aneuploidy focused on mutants in the Jimson weed, *Datura stramonium*. A. Francis Blakeslee began breeding this plant in 1913, and he observed that crosses with several Jimson mutants produced unusual ratios of progeny. For example, the globe mutant (having a seedcase globular in shape) was dominant but was inherited primarily from the female parent. When globe plants were self-fertilized, only 25% of the progeny had the globe phenotype, an unusual ratio for a dominant trait. Blakeslee isolated 12 different mutants that also exhibited peculiar patterns of inheritance. Eventually, John Belling demonstrated that these 12 mutants are in fact trisomics. *Datura stramonium* has 12 pairs of chromosomes (2n = 24), and each of the 12 mutants is trisomic for a different chromosome pair. The aneuploid nature of the mutants explained the unusual ratios that Blakeslee had observed in the progeny. Many of the extra chromosomes in the trisomics were lost in meiosis, so fewer than 50% of the gametes carried the extra chromosome, and the proportion of trisomics in the progeny was low. Furthermore, the pollen containing an extra chromosome was not as successful in fertilization, and trisomic zygotes were less viable.

Aneuploidy usually alters the phenotype drastically. In most animals and many plants, aneuploid mutations are lethal. Because aneuploidy affects the number of gene copies but not their nucleotide sequences, the effects of aneuploidy are most likely due to abnormal gene dosage.

Aneuploidy alters the dosage for some, but not all, genes, disrupting the relative concentrations of gene products and often interfering with normal development.

A major exception to the relation between gene number and protein dosage pertains to genes on the mammalian X chromosome. In mammals, X-chromosome inactivation ensures that males (who have a single X chromosome) and females (who have two X chromosomes) receive the same functional dosage for X-linked genes. Extra X chromosomes in mammals are inactivated; so we might expect that aneuploidy of the sex chromosomes would be less detrimental in these animals. Indeed, this is the case for mice and humans, for whom aneuploids of the sex chromosomes are the most common form of aneuploidy seen in living organisms. Y-chromosome aneuploids are probably common because there is so little information on the Y-chromosome.

Aneuploidy in Humans

Aneuploidy in humans usually produces serious developmental problems that lead to spontaneous abortion (miscarriage). In fact, as many as 50% of all spontaneously aborted fetuses carry chromosome defects, and a third or more of all conceptions spontaneously abort, usually so early in development that the mother is not even aware of her pregnancy. Only about 2% of all fetuses with a chromosome defect survive to birth.

Sex-chromosome aneuploids

The most common aneuploidy seen in living humans has to do with the sex chromosomes. As is true of all mammals, aneuploidy of the human sex chromosomes is better tolerated than aneuploidy of autosomal chromosomes. Turner syndrome and Klinefelter syndrome both result from aneuploidy of the sex chromosomes.

Autosomal aneuploids

Autosomal aneuploids resulting in live births are less common than sex-chromosome aneuploids in humans, probably because there is no mechanism of dosage compensation for autosomal chromosomes. Most autosomal aneuploids are spontaneously aborted, with the exception of aneuploids of some of the small autosomes.

Because these chromosomes are small and carry fewer genes, the presence of extra copies is less detrimental. For example, the most common autosomal aneuploidy in humans is trisomy 21, also called Down syndrome. The number of genes on different human chromosomes is not precisely known at the present time, but DNA sequence data indicate that chromosome 21 has fewer genes than any other autosome, with perhaps less than 300 genes of a total of 30,000 to 35,000 for the entire genome.

The incidence of Down syndrome in the United States is about 1 in 700 human births, although the incidence is higher among children born to older mothers. People with Down syndrome show variable degrees of mental retardation, with an average IQ of about 50 (compared with an average IQ of 100 in the general population).

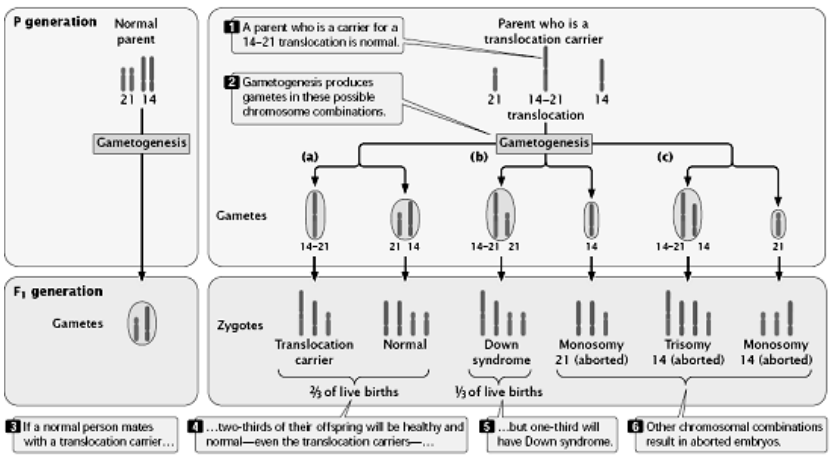
Many people with Down syndrome also have characteristic facial features, some retardation of growth and development, and an increased incidence of heart defects, leukemia, and other abnormalities.

Approximately 92% of those who have Down syndrome have three full copies of chromosome 21 (and therefore a total of 47 chromosomes), a condition termed primary Down syndrome. Primary Down syndrome usually arises from random non-disjunction in egg formation: about 75% of the non-disjunction events that cause Down syndrome are maternal in origin, and most arise in meiosis I. Most children with Down syndrome are born to normal parents, and the failure of the chromosomes to divide has little hereditary tendency. A couple who has conceived one child with primary Down syndrome has only a slightly higher risk of conceiving a second child with Down syndrome (compared with other couples of similar age who have not had any Down-syndrome children). Similarly, the couple's relatives are not more likely to have a child with primary Down syndrome. Most cases of primary Down syndrome arise from maternal nondisjunction, and the frequency of this occurring correlates with maternal age. Although the underlying cause of the association between maternal age and nondisjunction remains obscure, recent studies have indicated a strong correlation between nondisjunction and aberrant meiotic recombination. Most chromosomes that failed to separate in meiosis I do not show any evidence of having recombined with one another. Conversely, chromosomes that appear to have failed to separate in meiosis II often show evidence of recombination in regions that do not normally recombine, most notably near the centromere.

Although aberrant recombination appears to play a role in nondisjunction, the maternal age effect is more complex. In female mammals, prophase I begins in all oogonia during fetal development, and recombination is completed prior to birth. Meiosis then arrests in diplotene, and the primary oocytes remain suspended until just before ovulation. As each primary oocyte is ovulated, meiosis resumes and the first division is completed, producing a secondary oocyte. At this point, meiosis is suspended again, and remains so until the secondary oocyte is penetrated by a sperm. The second meiotic division takes place immediately before the nuclei of egg and sperm unite to form a zygote.

An explanation of the maternal age effect must take into account the aberrant recombination that occurs prenatally and the long suspension in prophase I. One theory is that the "best" oocytes are ovulated first, leaving those oocytes that had aberrant recombination to be used later in life. However, evidence indicates that the frequency of aberrant recombination is similar between oocytes that are ovulated in young women and those ovulated in older women. Another possible explanation is that aging of the cellular components needed for meiosis results in non-disjunction of chromosomes that are "at risk," because they have failed to recombine or had some recombination defect. In younger oocytes, these chromosomes can still be segregated from one another, but in older oocytes, they are sensitive to other perturbations in the meiotic machinery. In contrast, sperm are produced continually after puberty, with no long suspension of the meiotic divisions. This fundamental difference between the meiotic process in females and males may explain why most chromosome aneuploidy in humans is maternal in origin.

About 4% of people with Down syndrome have 46 chromosomes, but an extra copy of part of chromosome 21 is attached to another chromosome through a translocation (FIGURE 17). This condition is termed familial Down syndrome because it has a tendency to run in families. The phenotypic characteristics of familial Down syndrome are the same as those for primary Down syndrome. Familial Down syndrome arises in offspring whose parents are carriers of chromosomes that have undergone a Robertsonian translocation, most commonly between chromosome 21 and chromosome 14: the long arm of 21 and the short arm of 14 exchange places. This exchange produces a chromosome that includes the long arms of chromosomes 14 and 21, and a very small chromosome that consists of the short arms of chromosomes 21 and 14. The small chromosome is generally lost after several cell divisions.



17 Translocation carriers are at increased risk for producing children with Down syndrome.

Persons with the translocation, called translocation carriers, do not have Down syndrome. Although they possess only 45 chromosomes, their phenotypes are normal because they have two copies of the long arms of chromosomes 14 and 21, and apparently the short arms of these chromosomes (which are lost) carry no essential genetic information. Although translocation carriers are completely healthy, they have an increased chance of producing children with Down syndrome.

When a translocation carrier produces gametes, the translocation chromosome may segregate in three different ways. First, it may separate from the normal chromosomes 14 and 21 in anaphase I of meiosis. In this type of

segregation, half of the gametes will have the translocation chromosome and no other copies of chromosomes 21 and 14; the fusion of such a gamete with a normal gamete will give rise to a translocation carrier. The other half of the gametes produced by this first type of segregation will be normal, each with a single copy of chromosomes 21 and 14, and will result in normal offspring. Alternatively, the translocation chromosome may separate from chromosome 14 and pass into the same cell with the normal chromosome 21. This type of segregation produces all abnormal gametes; half will have two functional copies of chromosome 21 (one normal and one attached to chromosome 14) and the other half will lack chromosome 21. The gametes with the two functional copies of chromosome 21 will produce children with familial Down syndrome; the gametes lacking chromosome 21 will result in zygotes with monosomy 21 and will be spontaneously aborted.

In the third type of segregation, the translocation chromosome and the normal copy of chromosome 14 segregate together, and the normal chromosome 21 segregates by itself. This pattern is presumably rare, because the two centromeres are both derived from chromosome 14 separately from each other. In any case, all the gametes produced by this process are abnormal: half result in monosomy 14 and the other half result in trisomy 14— all are spontaneously aborted. Thus, only three of the six types of gametes that can be produced by a translocation carrier will result in the birth of a baby and, theoretically, these gametes should arise with equal frequency. One-third of the offspring of a translocation carrier should be translocation carriers like their parent, one-third should have familial Down syndrome, and one-third should be normal.

In reality, however, fewer than one-third of the children born to translocation carriers have Down syndrome, which suggests that some of the embryos with Down syndrome are spontaneously aborted.

Few autosomal aneuploids besides trisomy 21 result in human live births. Trisomy 18, also known as Edward syndrome, arises with a frequency of approximately 1 in 8000 live births. Babies with Edward syndrome are severely retarded and have low-set ears, a short neck, deformed feet, clenched fingers, heart problems, and other disabilities. Few live for more than a year after birth. Trisomy 13 has a frequency of about 1 in 15,000 live births and produces features that are collectively known as Patau syndrome. Characteristics of this condition include severe mental retardation, a small head, sloping forehead, small eyes, cleft lip and palate, extra fingers and toes, and numerous other problems. About half of children with trisomy 13 die within the first month of life, and 95% die by the age of 3. Rarer still is trisomy 8, which arises with a frequency of about 1 in 25,000 to 50,000 live births. This aneuploid is characterized by mental retardation, contracted fingers and toes, lowest malformed ears, and a prominent forehead. Many who have this condition have normal life expectancy.

Uniparental Disomy

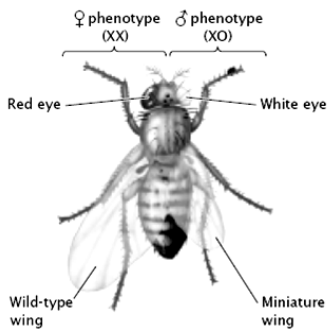
Normally, the two chromosomes of a homologous pair are inherited from different parents—one from the father and one from the mother. The development of molecular techniques that facilitate the identification of specific DNA sequences, has made it possible to determine the parental origins of chromosomes. Surprisingly, sometimes both chromosomes are inherited from the same parent, a condition termed uniparental disomy.

Uniparental disomy violates the rule that children affected with a recessive disorder appear only in families where both parents are carriers. For example, cystic fibrosis is an autosomal recessive disease; typically, both parents of an affected child are heterozygous for the cystic fibrosis mutation on chromosome 7. However, a small proportion of people with cystic fibrosis have only a single parent who is heterozygous for the cystic fibrosis gene. How can this be? These people must have inherited from the heterozygous parent two copies of the chromosome 7 that carries the defective cystic fibrosis allele and no copy of the normal allele from the other parent. Uniparental disomy has also been observed in Prader-Willi syndrome, a rare condition that arises when a paternal copy of a gene on chromosome 15 is missing. Although most cases of Prader-Willi syndrome result from a chromosome deletion that removes the paternal copy of the gene, from 20% to 30% arise when both copies of chromosome 15 are inherited from the mother and no copy is inherited from the father.

Many cases of uniparental disomy probably originate as a trisomy. Although most autosomal trisomies are lethal, a trisomic embryo can survive if one of the three chromosomes is lost early in development. If, just by chance, the two remaining chromosomes are both from the same parent, uniparental disomy results.

Mosaicism

Nondisjunction in a mitotic division may generate patches of cells in which every cell has a chromosome abnormality and other patches in which every cell has a normal karyotype. This type of nondisjunction leads to regions of tissue with different chromosome constitutions, a condition known as mosaicism. Growing evidence suggests that mosaicism is relatively common. Only about 50% of those diagnosed with Turner syndrome have the 45,X karyotype (presence of a single X chromosome) in all their cells; most others are mosaics, possessing some 45,X cells and some normal 46,XX cells. A few may even be mosaics for two or more types of abnormal karyotypes. The 45,X/46,XX mosaic usually arises when an X chromosome is lost soon after fertilization in an XX embryo.



18 Mosaicism for the sex chromosomes produces a gynandromorph. This XX/XO

Fruit flies that are XX/XO mosaics (O designates the absence of a homologous chromosome; XO means the cell has a single X chromosome and no Y chromosome) develop a mixture of male and female traits, because the presence of two X chromosomes in fruit flies produces female traits and the presence of a single X chromosome produces male traits (FIGURE 18). Sex determination in fruit flies occurs independently in each cell during development. Those cells that are XX express female traits; those that are XY express male traits. Such sexual mosaics are called gynandromorphs. Normally, X-linked recessive genes are masked in heterozygous females but, in XX/XO mosaics, any X-linked recessive genes present in the cells with a single X chromosome will be expressed.

Polyploidy

Most eukaryotic organisms are diploid (2n) for most of their life cycles, possessing two sets of chromosomes. Occasionally, whole sets of chromosomes fail to separate in meiosis or mitosis, leading to polyploidy, the presence of more than two genomic sets of chromosomes. Polyploids include triploids (3n) tetraploids (4n), pentaploids (5n), and even higher numbers of chromosome sets. Polyploidy is common in plants and is a major mechanism by which new plant species have evolved.

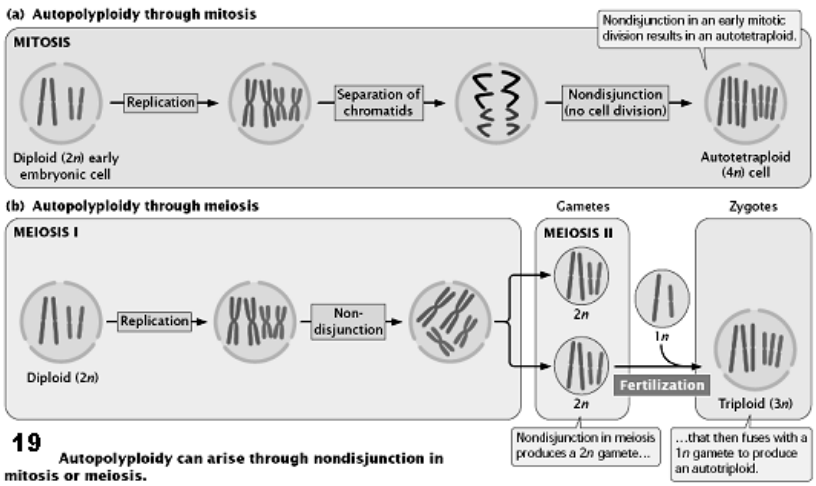
Approximately 40% of all flowering-plant species and from 70% to 80% of grasses are polyploids. They include a number of agriculturally important plants, such as wheat, oats, cotton, potatoes, and sugar cane. Polyploidy is less common in animals, but is found in some invertebrates, fishes, salamanders, frogs, and lizards. No naturally occurring, viable polyploids are known in birds, but at least one polyploid mammal—a rat from Argentina— has been reported.

We will consider two major types of polyploidy: autopolyploidy, in which all chromosome sets are from a single species; and allopolyploidy, in which chromosome sets are from two or more species.

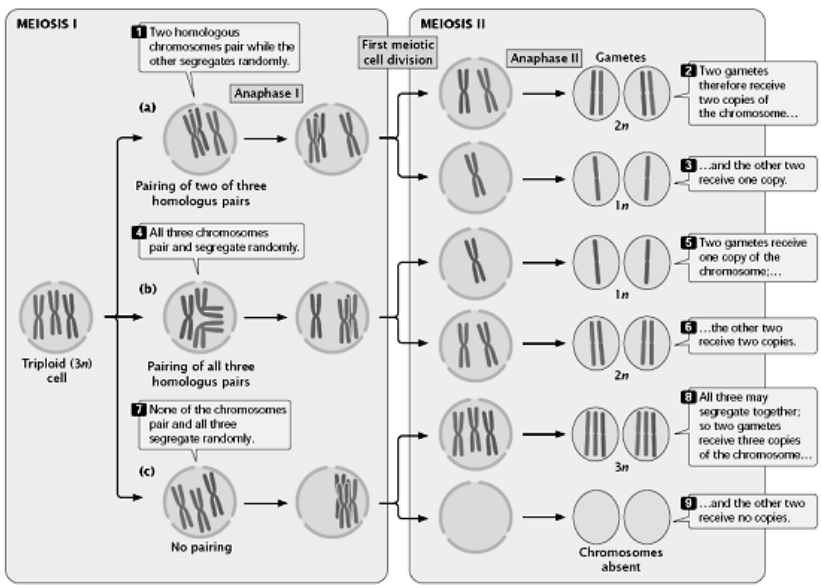
Autopolyploidy

Autopolyploidy results when accidents of meiosis or mitosis produce extra sets of chromosomes, all derived from a single species. Nondisjunction of all chromosomes in mitosis in an early 2n embryo, for example, doubles the chromosome number and produces an autotetraploid (4n) (FIGURE 19a). An autotriploid may arise when nondisjunction in meiosis produces a diploid gamete that then fuses with a normal haploid gamete to produce a triploid zygote (FIGURE 19b). Alternatively, triploids may arise from a cross between an autotetraploid that produces 2n gametes and a diploid that produces 1n gametes. Because all the chromosomes sets in autopolyploids are from the same species, they are homologous and attempt to align in prophase I of meiosis, which usually result in sterility.

Because all the chromosome sets in autopolyploids are from the same species, they are homologous and attempt to align in prophase I of meiosis, which usually results in sterility. Consider meiosis in an autotriploid (FIGURE 20). In meiosis in a diploid cell, two chromosome homologs pair and align, but, in autotriploids, three homologs are present. One of the three homologs may fail to align with the other two, and this unaligned chromosome will segregate randomly (see Figure 20a).Which gamete gets the extra chromosome will be determined by chance and will differ for each homologous group of chromosomes. The resulting gametes will have two copies of some chromosomes and one copy of others. Even if all three chromosomes do align, two chromosomes must segregate to one gamete and one chromosome to the other (see Figure 20b). Occasionally, the presence of a third chromosome interferes with normal alignment, and all three chromosomes segregate to the same gamete (see Figure 20c).



19 **Autopolyploidy can arise through nondisjunction in mitosis or meiosis.**



20 **In meiosis of an autotriploid, homologous chromosomes can pair or not pair in three ways.**

No matter how the three homologous chromosomes align, their random segregation will create unbalanced gametes, with various numbers of chromosomes. A gamete produced by meiosis in such an autotriploid might receive, say, two copies of chromosome 1, one copy of chromosome 2, three copies of chromosome 3, and no copies of chromosome 4. When the unbalanced gamete fuses with a normal gamete (or with another unbalanced gamete), the resulting zygote has different numbers of the four types of chromosomes. This difference in number creates unbalanced gene dosage

in the zygote, which is often lethal. For this reason, triploids do not usually produce viable offspring.

In even-numbered autopolyploids, such as autotetraploids, it is theoretically possible for the homologous chromosomes to form pairs and divide equally. However, this event rarely happens; so these types of autotetraploids also produce unbalanced gametes.

The sterility that usually accompanies autopolyploidy has been exploited in agriculture. Wild diploid bananas (2n = 22), for example, produce seeds that are hard and inedible, but triploid bananas (3n = 33) are sterile, and produce no seeds—they are the bananas sold commercially. Similarly, seedless triploid watermelons have been createdand are now widely sold.

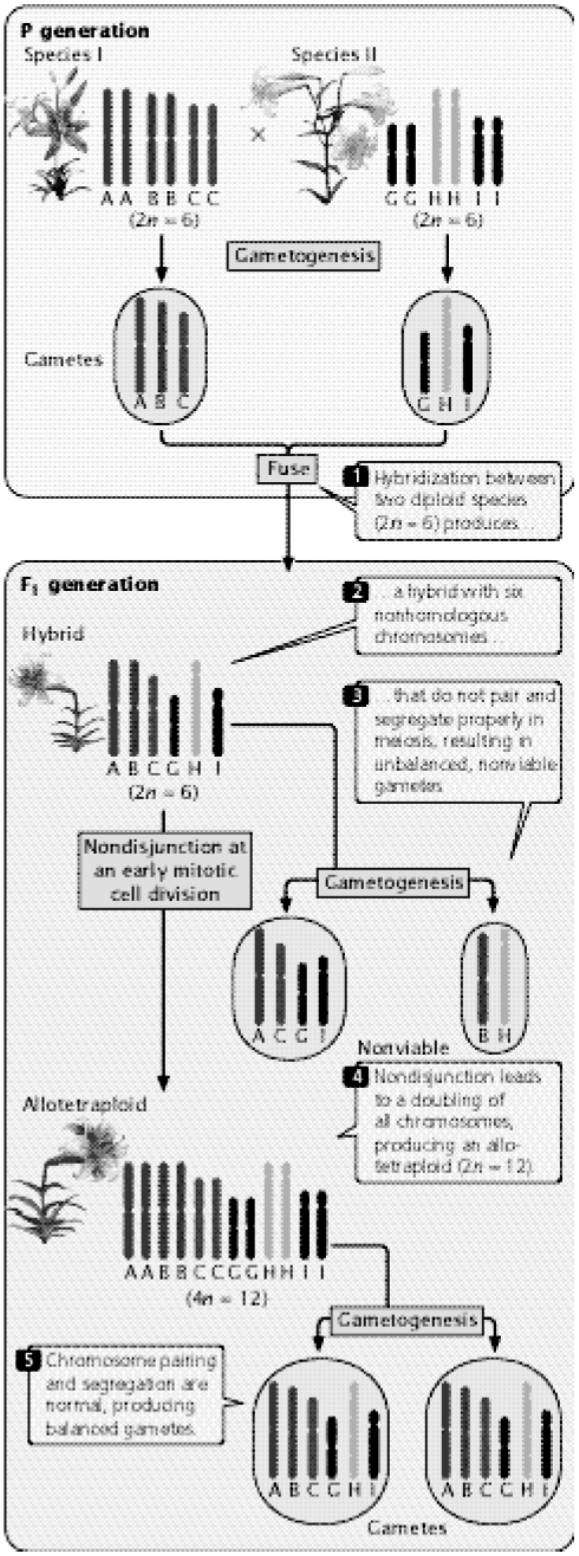
Allopolyploidy

Allopolyploidy arises from hybridization between two species; the resulting polyploid carries chromosome sets derived from two or more species. FIGURE 21 shows how alloploidy can arise from two species that are sufficiently related that hybridization occurs between them. Species I (AABBCC, 2n = 6) produces haploid gametes with chromosomes ABC, and species II (GGHHII, 2n = 6) produces gametes with chromosomes GHI.

If gametes from species I and II fuse, a hybrid with six chromosomes (ABCGHI) is created. The hybrid has the same chromosome number as that of both diploid species; so the hybrid is considered diploid. However, because the hybrid chromosomes are not homologous, they will not pair and segregate properly in meiosis; so this hybrid is functionally haploid and sterile.

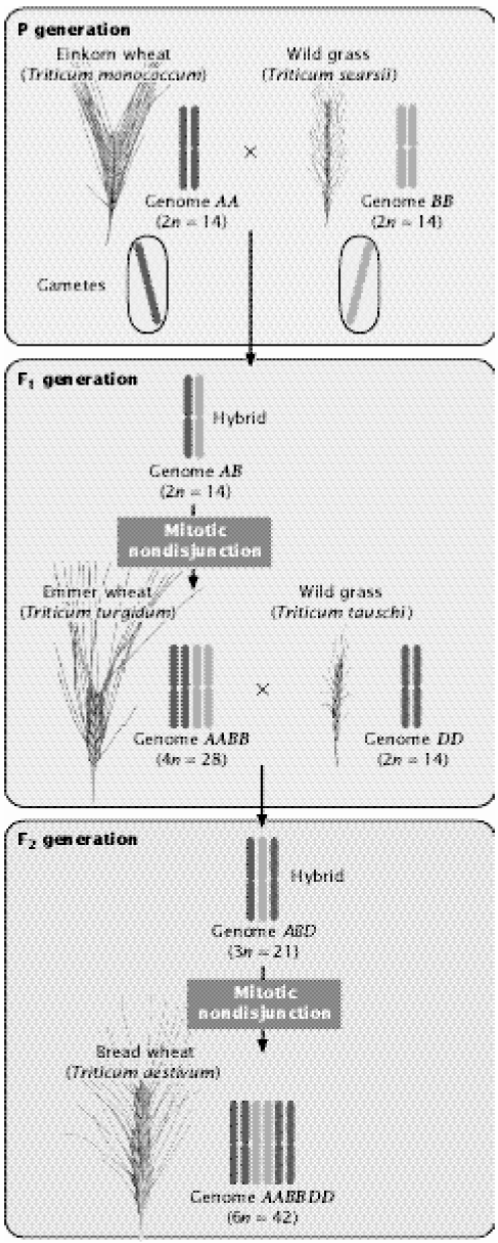
The sterile hybrid is unable to produce viable gametes through meiosis, but it may be able to perpetuate itself through mitosis (asexual reproduction). On rare occasions, nondisjunction takes place in a mitotic division, which leads to a doubling of chromosome number and an allotetraploid, with chromosomes AABBCCGGHHII. This tetraploid is functionally diploid: every chromosome has one and only one homologous partner, which is exactly what meiosis requires for proper segregation. The allopolyploid can now undergo normal meiosis to produce balanced gametes having six chromosomes. George Karpechenko created polyploids experimentally in the 1920s. Today, as well as in the early twentieth century, cabbage (Brassica oleracea, 2n = 18) and radishes (Raphanus sativa, 2n = 18) are agriculturally important plants, but only the leaves of the cabbage and the roots of the radish are normally consumed. Karpechenko wanted to produce a plant that had cabbage leaves and radish roots so that no part of the plant would go to waste. Because both cabbage and radish possess 18 chromosomes, Karpechenko was able to successfully cross them, producing a hybrid with 2n = 18, but, unfortunately, the hybrid was sterile. After several crosses, Karpechenko noticed that one of his hybrid plants produced a few seeds.

When planted, these seeds grew into plants that were viable and fertile. Analysis of their chromosomes revealed that the plants were allotetraploids, with 2n = 36 chromosomes. To Karpechencko's great disappointment, however, the new plants possessed the roots of a cabbage and the leaves of a radish.



The Significance of Polyploidy

In many organisms, cell volume is correlated with nuclear volume, which, in turn, is determined by genome size. Thus, the increase in chromosome number in polyploidy is often associated with an increase in cell size, and many polyploids are physically larger than diploids. Breeders have used this effect to produce plants with larger leaves, flowers, fruits, and seeds. The hexaploid (6n = 42) genome of wheat probably contains chromosomes derived from three different wild species (FIGURE 22).Many other cultivated plants also are polyploid (Table 2).



Polyploidy is less common in animals than in plants for several reasons. As discussed, allopolyploids require hybridization between different species, which occurs less frequently in animals than in plants.

Table 2 Examples of polyploid crop plants

Plant	Type of Polyploidy	Ploidy	Chromosome Number
Potato	Autopolyploid	4*n*	48
Banana	Autopolyploid	3*n*	33
Peanut	Autopolyploid	4*n*	40
Sweet potato	Autopolyploid	6*n*	90
Tobacco	Allopolyploid	4*n*	48
Cotton	Allopolyploid	4*n*	52
Wheat	Allopolyploid	6*n*	42
Oats	Allopolyploid	6*n*	42
Sugar cane	Allopolyploid	8*n*	80
Strawberry	Allopolyploid	8*n*	56

Animal behavior often prevents interbreeding, and the complexity of animal development causes most interspecific hybrids to be nonviable. Many of the polyploid animals that do arise are in groups that reproduce through parthenogenesis (a type of reproduction in which individuals develop from unfertilized eggs). Thus asexual reproduction may facilitate the development of polyploids, perhaps because the perpetuation of hybrids through asexual reproduction provides greater opportunities for nondisjunction. Only a few human polyploid babies have been reported, and most died within a few days of birth. Polyploidy—usually triploidy—is seen in about 10% of all spontaneously aborted human fetuses. Different types of chromosome mutations are summarized in Table 3.

Table 3 Different types of chromosome mutations

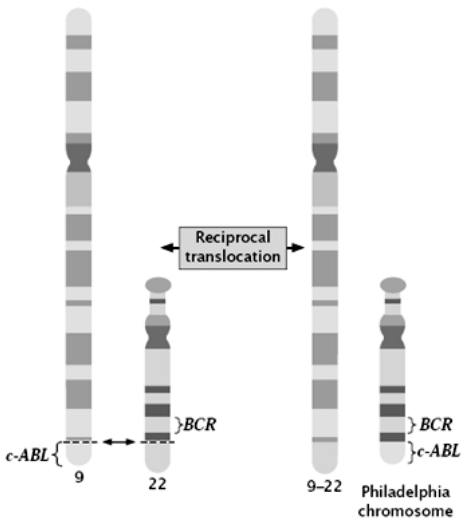
Chromosome Mutation	Definition
Chromosome rearrangement	Change in chromosome structure
Chromosome duplication	Duplication of a chromosome segment
Chromosome	Deletion of a chromosome segment
Inversion	Chromosome segment inverted 180 degrees
Paracentric inversion	Inversion that does not include the centromere in the inverted region
Pericentric inversion	Inversion that includes the centromere in the inverted region
Translocation	Movement of a chromosome segment to a nonhomologous chromosome or region of the same chromosome
Nonreciprocal translocation	Movement of a chromosome segment to a nonhomologous chromosome or region of the same chromosome without reciprocal exchange
Reciprocal translocation	Exchange between segments of nonhomologous chromosomes or regions of the same chromosome
Aneuploidy	Change in number of individual chromosomes
Nullisomy	Loss of both members of a homologous pair
Monosomy	Loss of one member of a homologous pair
Trisomy	Gain of one chromosome, resulting in three homologous chromosomes
Tetrasomy	Gain of two homologous chromosomes, resulting in four homologous chromosomes
Polyploidy	Addition of entire chromosome sets
Autopolyploidy	Polyploidy in which extra chromosome sets are derived from the same species
Allopolyploidy	Polyploidy in which extra chromosome sets are derived from two or more species

Chromosome Mutations and Cancer

Most tumors contain cells with chromosome mutations. For many years, geneticists argued about whether these chromosome mutations were the cause or the result of cancer. Some types of tumors are consistently associated with specific chromosome mutations, suggesting that in these cases the specific chromosome mutation played a pivotal role in the development of the cancer. However,

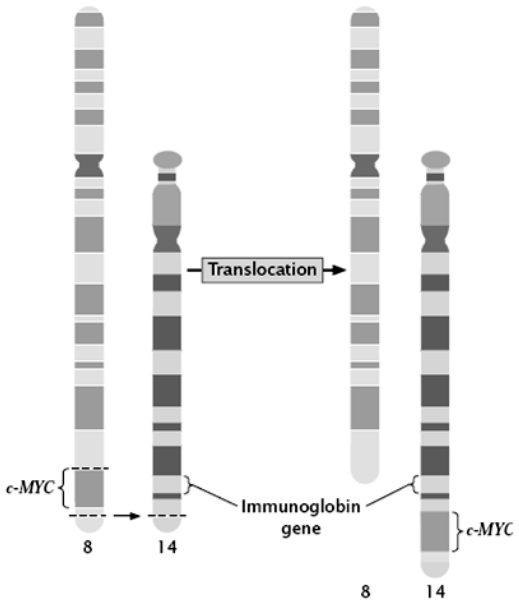
many cancers are not associated with specific types of chromosome abnormalities, and individual gene mutations are now known to contribute to many types of cancer. Nevertheless, chromosome instability is a general feature of cancer cells, causing them to accumulate chromosome mutations, which then affect individual genes that contribute to the cancer process. Thus, chromosome mutations appear to both cause and be a result of cancer. At least three types of chromosome rearrangements— deletions, inversions, and translocations—are associated with certain types of cancer. Deletions may result in the loss of one or more genes that normally hold cell division in check. When these so-called tumor-suppressor genes are lost, cell division is not regulated and cancer may result.

Inversions and translocations contribute to cancer in several ways. First, the chromosomal breakpoints that accompany these mutations may lie within tumor-suppressor genes, disrupting their function and leading to cell proliferation. Second, translocations and inversions may bring together sequences from two different genes, generating a fused protein that stimulates some aspect of the cancer process. Such fusions are seen in most cases of chronic myeloid leukemia, a fatal form of leukemia affecting bone marrow cells. About 90% of patients with chronic myeloid leukemia have a reciprocal translocation between the long arm of chromosome 22 and the tip of the long arm of chromosome 9 (FIGURE 23).



23 A reciprocal translocation between chromosomes 9 and 22 causes chronic myeloid leukemia.

This translocation produces a shortened chromosome 22, called the Philadelphia chromosome because it was first discovered in Philadelphia. At the end of a normal chromosome 9 is a potential cancer-causing gene called c-ABL. As a result of the translocation, part of the c-ABL gene is fused with the BCR gene from chromosome 22. The protein produced by this BCR–c-ABL fusion gene is much more active than the protein produced by the normal c-ABL gene; the fusion protein stimulates increased, unregulated cell division and eventually leads to leukemia. A third mechanism by which chromosome rearrangements may produce cancer is by the transfer of a potential cancer-causing gene to a new location, where it is activated by different regulatory sequences. Burkitt lymphoma is a cancer of the B cells, the lymphocytes that produce antibodies. Many people having Burkitt lymphoma possess a reciprocal translocation between chromosome 8 and chromosome 2, 14, or 22, each of which carries genes for immunological proteins (FIGURE 24). This translocation relocates a gene called c-MYC from the tip of chromosome 8 to a position in one of the aforementioned chromosomes that is next to a gene for one of the immunoglobulin proteins.



24 A reciprocal translocation between chromosomes 8 and 14 causes Burkitt lymphoma.

At this new location, c-MYC comes under the control of regulatory sequences that normally activate the production of immunoglobulins, and c-MYC is expressed in B cells. The c-MYC protein stimulates the division of the B cells and leads to Burkitt lymphoma.

K. Recombination: Homologous and non-homologous recombination, including transposition, site-specific recombination.

I. INTRODUCTION

A. Biological Significance of Mobile DNA Elements

The development of and interest in the idea nature of mobile genetic elements Barbara McClintock's seminar papers describing eukaryotic transposition of what she called controlling elements in maize during the 1950s. McClintock demonstrated that movement of specific genetic elements to new chromosomal locations affecting the expression of nearby genes and caused chromoso,al breakages in a developmentally regulated manner. Since McClintock's remarkable discovery, the dynamic nature of mobile genetic elements has been observed in almost every prokaryotic and eukaryotic organisms investigated to date.

The genome of any organism must possess two key features. First it must be stable enough to pass accurate information through inheritance, ensuring the survival of progeny.

However, the genome must also be dynamic in order to respond to selective environmental pressures. Therefore any successful biological system must maintain a delicate balance between genome integrity and flexibility. In both prokaryotic and eukaryotic systems, recombination is one of the key mechanisms that regulates genome integrity. Chromosomal breakages and mutations, stemming from problems in DNA replication or environmental stress, can be repaired through recombination pathways. In some cases organisms contain multiple recombination pathways by which damage can be repaired, underscoring the importance of this process.

The mobility of DNA sequence elements is also a driving force in evolutionary patterns. Recombination provides a mechanism by which DNA can be moved, deleted and amplified to effect these changes. These movements are often tightly regulated, such as those involved in gene rearrangements, DNA amplification and deletion, and genome integration events. In other examples, the movements are rare and only become visible when selective pressures are imposed on large populations. One of the most startling examples of recombination-driven evolution involves the inheritance of antibiotic resistance genes among certain populations of bacteria. The biological consequences of recombination are ubiquitous.

In recent years recombination has become an invaluable tool in the biological laboratory for both genome manipulation and genetic analysis. Understanding mechanisms of recombination is therefore central to any discussion of the functional properties of any particular genome. Moreover recombination systems offer the opportunity to harness the power of mobile DNA elements for use in genetic therapies and other medical applications.

B. Genetic Recombination: Background and Perspective

The modern field of recombination, by most accounts, began in 1964 with Robin Holliday's hypothetical four-stranded DNA structure (Holliday, 1964) (Fig. 1). This structure, later to be named the Holliday junction, consists of two DNA duplexes associated by a single-stranded crossover and was proposed to explain gene conversions previously observed in fungi. Holliday possessed one advantage over McClintock in her earlier work: knowledge of the structure of DNA. Knowing that DNA was double stranded, he proposed a mechanism by which two chromosomes in close proximity could exchange DNA strands, thereby effecting genomic alterations. He also noted the necessity for homology between the exchanging strands. Historically the Holliday junction and the necessity for extensive DNA homology have defined classical recombination, but it is important to note that there are many different recombination reaction mechanisms that proceed through a variety of intermediate structures.

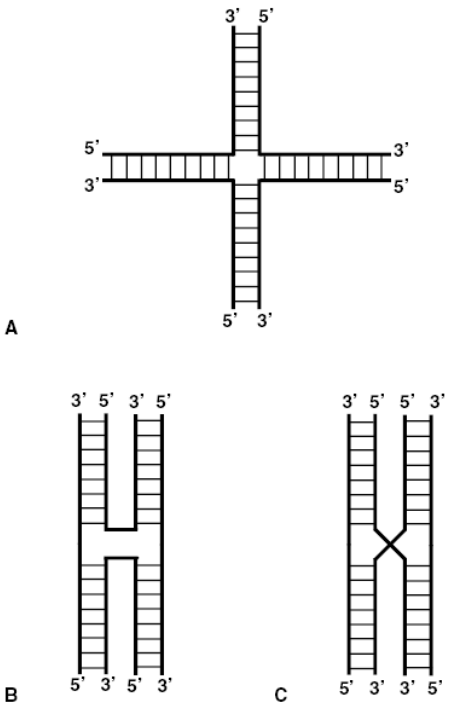


Fig. 1. Plane projections of three different structures of a four-way DNA junction, or Holliday junction. **A:** Symmetric planar cross. **B:** Antiparallel, open junction; **C:** Crossed, parallel junction.

II. RECOMBINATION SYSTEMS

A. General or Homologous Recombination

The most familiar recombination systems are those that are involved in general or homologous recombination. This class of recombination events is defined by the exchange of homologous sequences between double-stranded DNA molecules and results in recombinant product molecules that contain genetic information originally present in each of the parental molecules. There is generally no limit to how much DNA can be exchanged in this process, ranging from

tens to thousands of base pairs, so long as homology is maintained between recombining duplexes. The basis of recognition in homologous systems is pairing of the exchanging DNA sequences. Protein components of these systems have other roles in the recombination reaction such as DNA strand juxtaposition, recruitment of cofactors such as ATP or accessory proteins, catalysis of strand cleavage and rejoining reactions, and heteroduplex extension or branch migration.

A key intermediate in general recombination pathways is the four-stranded Holliday junction (Fig. 1). One of the hallmarks of this four-way junction is its ability to undergo branch migration, which can extend the heteroduplex region for thousands of base pairs (Fig. 2). Branch migration can occur spontaneously by thermal fluctuations, resulting in unidimensional diffusion of the junction's branch point, or can be driven by helicase-dependent ATP hydrolysis. The final step of homologous recombination involves resolution of the four-way junction into two distinct duplex molecules. Resolution in homologous systems can occur in a variety of ways, depending on the specificity of resolving enzymes and the particular conformation of the DNA intermediate. The locations of strand cleavages that resolve the junction direct the formation of particular products. Homologous recombination systems are involved in numerous cellular functions, including the repair of genomic damage caused by mismatched base pairs, chromosomal breaks, or deletions, mating-type conversion, antigenic variation, DNA replication, and meiosis.

B. Homologous Recombination in *Escherichia coli*

The paradigm for general recombination is abstracted from the set of RecA-dependent homologous recombination systems of E. coli. Although our understanding of other homologous recombination systems in both prokaryotic and eukaryotic organisms is rapidly improving, none of these approach the extent to which the RecA-dependent pathway has been characterized. At least 25 proteins have been shown to play some role in all types of homologous recombination in E. coli; many of these proteins have functional homologs in other organisms though there is often little structural homology.

The strand-exchange protein RecA plays a central role in nearly all E. coli homologous recombination pathways. The conservation of functional RecA homologs underscores the biological importance of DNA strand-exchange proteins: all free-living organisms examined to date possess a RecA-like protein. RecA-dependent homologous recombination involves at least four distinct stages (Fig. 2):

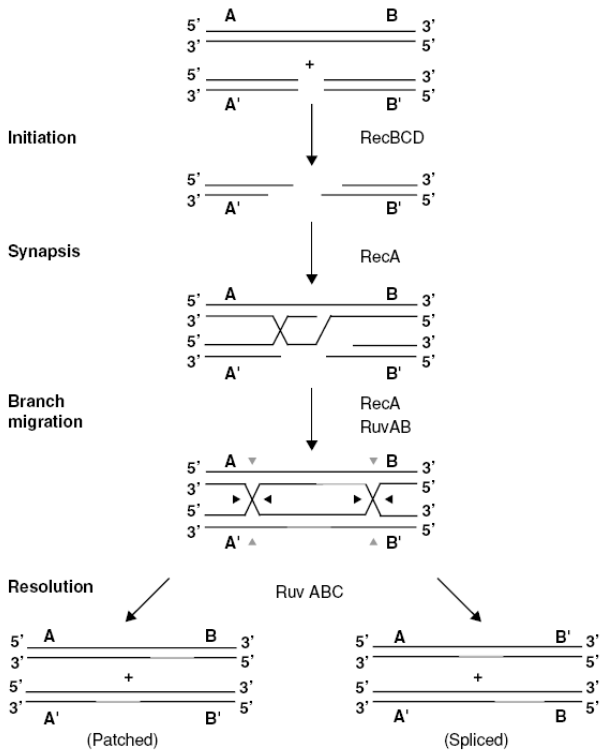


Fig. 2. Model for general or homologous recombination in *E. coli*. Stages of recombination are shown in bold; enzyme systems associated with each step are also given. Letters indicating the locations of arbitrary DNA sequence elements are given for reference. Initiation occurs at a double-stranded break and involves resection of 3′ single-stranded overhangs by RecBCD. Synapsis and strand invasion by one of the processed ends into the intact duplex is mediated by RecA protein to form a D-loop structure. DNA synthesis then extends the free 3′ ends to form a double Holliday-junction structure; newly synthesized DNA is shown in gray. Both Holliday junctions undergo branch migration promoted by RecA and RuvAB. Resolution of the junctions by the RuvABC complex involves a second set of strand-exchange steps and can take place in either of two planes through each Holliday junction. Resolution that occurs via strand exchange at both sets of dark arrowheads or both sets of light arrowheads generates recombinant products shown at bottom left (patched products). In contrast, resolution at opposing combinations of orientations, one set of dark arrowheads and one set of light arrowheads, yields recombinant products shown at bottom right (spliced products). (Adapted

1. Initiation

Initiation encompasses the processing of DNA at a double-stranded break to generate a single-stranded DNA segment required for strand invasion of a duplex DNA homolog by a RecA-ssDNA complex. The DNA processing that occurs during initiation in E. coli is carried out by the recombination-specific helicases RecBCD and RecQ, probably in conjunction with some exogenous exonuclease activity, such as that of the protein RecJ. In addition to helicase activity, the RecBCD complex also has an intrinsic nuclease activity; the specificity of this nuclease -activity is modulated dramatically by the presence of an 8 bp DNA sequence element, χ, which functions as a hotspot for homologous recombination . Upon encountering a χ site in the appropriate orientation, RecBCD switches from a 3'→5' to a 5'→ 3' exonuclease activity. This strand-polarity switch in the exonuclease activity of RecBCD leads to the preferential formation of DNA molecules bearing a 3'-terminal single-stranded overhang, which are ideal substrates for formation of a RecA-ssDNA filament known as the presynaptic filament.

2. Synapsis

This stage involves steps that lead to homologous pairing and strand exchange. In the presence of ATP or non hydrolyzable ATP analogues, RecA protein binds in a highly cooperative manner to single-stranded DNA to produce a nucleoprotein filament in which the ssDNA is stretched to nearly 1.5 times its original length. Both in vitro and in vivo, binding of RecA to ssDNA is assisted by E. coli single-stranded binding protein (SSB), which facilitates RecA binding through the destabilization of internal DNA secondary structure. Although ATPbinding stabilizes the ssDNA-specific form of RecA, ATPhydrolysis is not required either for formation of the presynaptic filament or for homologous pairing or strand exchange.

Synapsis occurs between the presynaptic filament, which contains the invading segment of single-stranded DNA, and a double-stranded homologous target sequence. Binding of the presynaptic filament to dsDNA is initially random, but the RecAssDNA filament carries out a rapid and efficient search for sequence homology by a mechanism not fully understood at present. Upon locating the homologous target sequence, the RecA-DNA filament generates what is known as a joint molecule, in which the invading single strand displaces the complementary strand of the duplex to form a D loop. Current structural and biochemical data overwhelmingly support a model for a joint molecule-RecA complex that contains three DNA strands; this view is in contrast to previously proposed models that invoke a four-stranded DNA intermediate . Thus it seems likely that the homology-search mechanism involves weak and transient binding of the target dsDNA to a secondary binding site on the presynaptic filament. Upon locating the homologous region, the RecAssDNA filament and/or double-stranded target sequence likely undergo conformational rearrangements that transfer the displaced complementary strand to the secondary DNA-binding site. However, the joint molecule remains relatively unstable subject to additional processing events.

Joint-molecule recombination intermediates are stabilized by the formation of the Holliday junction, which requires both DNA synthesis and strand-joining activities. The mechanistic details of this process remain largely unknown, although both polymerase I and topoisomerase I activities have been implicated in this step.

3. Branch migration

Assembly of RecA filaments on ssDNA occurs exclusively in a 5^0! 3^0 direction. This activity probably continues on joint molecules, advancing the branch point of the Holliday junction in the same direction with respect to the incoming DNA strand at a rate of about 6 nt sec^{-1} . However, because branch-migration activity seems to be largely bidirectional, the RuvAB helicase complex is thought to be the principal factor involved in this phase of homologous recombination. RuvA protein binds to the Holliday junction and recruits RuvB, the latter assembling into a typical ringlike, hexameric helicase structure that surrounds each of two duplex branches of the Holliday intermediate. These two helicase structures translocate the duplex DNA in opposing directions, causing DNA to be "pumped out'' of the center of the junction, thereby facilitating branch migration. Although branch-migration proceeds at a rate comparable to that promoted by RecA-binding activity, RuvAB catalyzes branch migration bidirectionally depending on which pair of duplex Holliday-junction arms are bound by the RuvB hexamers.

4. Resolution

The Holliday junction is specifically cleaved by the endonuclease activity of RuvC. The RuvC endonuclease is highly specific for Holliday junctions, and its cleavage activity occurs in concert with the branch-migration activity of RuvAB, presumably to locate RuvC at preferred cleavage sites. RuvC is capable of cleaving the Holliday junction in either of two ways leading to two potential sets of resolution products (Fig. 2). However, protein-DNA interactions probably distort the structure of the junction and thereby generate a preference for one of these.

C. Site-Specific Recombination

Unlike general recombination, site-specific recombination events involve the interaction of defined DNA sequence elements. These sequences are highly specialized, carry specific binding sites for the recombination proteins as well as the point of genetic exchange, and are usually present in extremely low copy number in the genome. Often these sites are present in pairs. However, they are sometimes present only as a single copy as in the case of the bacteriophage l integration site in the E. coli genome. This extraordinary degree of specificity leads to precisely defined genetic rearrangements. In the examples considered here, the rearrangements that occur are essentially uniquely defined.

Another important attribute of a site-specific recombination locus is the polarity of the recombination site. These loci are frequently nonpalindromic and therefore have an intrinsic polarity. Recombination normally occurs only when a pair of recombination sites has been juxtaposed in a particular spatial alignment, thereby imparting both positional and orientational specificity to these systems (Gellert and Nash,

1987; Nash, 1996). This specificity has important biological consequences; moreover the site-orientation specificity leads to the formation of specific DNA topologies in the recombination products. The topological specificity of site-specific recombination systems has been exploited to great effect in unraveling the mechanisms of many site-specific recombinases.

DNA homology normally plays a very limited role in site-specific recombination, more a feature of specific recombinase-DNA interactions than a necessity for homologous pairing or strand exchange. Unlike virtually all other modes of recombination, site-specific recombination is conservative in that no DNA is gained or lost during the recombination reaction. This aspect of site-specific recombination applies both at the level of genetic information (recombination products are merely permutations of the original parental DNA) and at the level of actual DNA nucleotides (no DNA synthesis or nucleolytic degradation is involved). In contrast, significant levels of DNA synthesis activity are required both for homologous recombination (see above) and transposition (see below).

Initiation of site-specific recombination begins with the binding of the recombination proteins to their respective recognition sequences within recombining loci. Upon binding to the target sites, protein-protein interactions among the recombination proteins facilitate the synapsis of recombination sites. Well-defined protein-DNA contacts allow site-specific recombinases to cleave their DNA targets with the specificity of restriction endonucleases, whereas protein-protein interactions direct strand exchange. An early step in virtually all site-specific recombination pathways is the formation of a covalently linked protein-DNA intermediate during the initial strand-cleavage reaction.

All site-specific recombination systems that have been investigated to date fall into two superfamilies: the integrase and resolvase/invertase families. Particular examples from both of these families are discussed below. Products of reactions carried out by the integrase superfamily vary widely depending on the orientation and disposition of recombination sites; this variability permits systems such as l-integrase to participate in both integrative and excisive recombination in a highly regulated fashion. The resolvase/invertase mechanisms are characterized by a well-defined DNA geometry in the synaptic intermediate and, as a consequence, tightly controlled product topologies. The two superfamilies are also distinct in terms of the intermediate structure of the DNA segments undergoing recombination; whereas λ-integrase-type mechanisms proceed through a Holliday intermediate, the resolvase/invertase mechanisms do not. Site-specific recombination systems participate in a wide range of biological processes in both prokaryotes and eukaryotes: viral integration, antigenic variation, gene duplication and copy-number control, and the integration of antibiotic resistance cassettes.

1. Integrative and excisive recombination in the λ-integrase system

The λ-integrase (λ-int) system is vital to the lysogenic stage of the life cycle of bacteriophage λ and is one of the most intensively studied site-specific recombination systems. A notable feature of this system is the nonsymmetrical nature of the integrative and excisive recombination reactions: although strand exchange activities are identical both for integration and excision of the phage-λ genome, each reaction has distinct requirements for specific DNA sequences at the recombining loci and subsets of protein cofactors involved in recombination.

Integration of phage λ occurs at a unique 25 bp site, termed attB, on the 4.6 Mbp *E. coli* chromosome. The catalytic activity for strand exchange resides in the l-encoded integrase protein (int), which functions in concert with a number of DNA-binding accessory proteins: the integration host factor (IHF) and factor for inversion stimulation (FIS) proteins of E. coli, and the λ-excisionase (Xis), which is phage-encoded. In contrast to the attB site, which by itself has negligible affinity for the recombination proteins, the recombination locus on the phage genome, attP, is about 250 bp in size and has multiple binding sites for int and the accessory factors. Integrative recombination most likely involves assembly of int and IHF proteins to form an organized nucleoprotein structure called the intosome, which subsequently captures a protein-free attB site during synapsis . Products of the integrative recombination reaction are a functionally distinct pair of new recombination sites, called attL and attR, that are no longer competent to participate in subsequent rounds of integrative recombination . Instead, these sites are substrates for excisive recombination, a reaction that requires FIS and Xis in addition to int and IHF. By coupling recombination to intracellular levels of specific protein factors, tight regulation of the phage-λ life cycle can be achieved in vivo.

D. Transposition

Transposable elements are mobile segments of DNA that can insert into nonhomologous target sites. The first prokaryotic element was discovered by Taylor in bacteriophage Mu, which was so named because of the mutations it caused in its *E. coli* host. Taylor observed that mu did not have a specific attachment site like phage l, but was inserted almost randomly, causing mutations in genes and regulatory regions that had been disrupted . Transposable elements may carry noncoding segments of DNA, the genome of a virus or phage, or antibiotic resistance elements. However, essential features of these elements are (1) they encode at least one protein factor that is involved in insertion, the transposase, and (2) the presence of terminal sequences that are recognized by the transposase and function as donor recombination sites. The transposase binds specifically to the end sequences, and either alone or in conjunction with accessory proteins, it is generally responsible for target-site selectivity. Transposon-end sequences are specific to each element and are frequently identical or consensus sequences that are arranged as a pair of terminal inverted repeats.
Target specificity varies widely among transposition systems and is characterized by avoidance of particular loci in the targeted genome as much as expressed preferences for particular sites of integration. Target-site specificity ranges from very weak, as in the case of phage mu, to moderate (e.g., Tn10, IS10), to high (e.g. Tn7); for some classes of transposable elements there is a weak consensus that corresponds to preferential insertion in A +T-rich

regions, but this is not a universal characteristic. In some cases a sequence-dependent structure such as an intrinsic DNA bend is targeted rather than a particular sequence, per se. A low degree of target specificity suggests that transposition reactions must be tightly regulated in order to prevent the accumulation of excessive mutation by the host genome.

Transposable elements are divided into two general classes: transposons, which use a DNA intermediate for direct insertion, and retrotransposons, which proceed through an RNA intermediate. Within these classes mechanistic details vary widely. However, a universal feature is that transposons are directly inserted into the target site by a series of DNA cleavage and strand-transfer reactions mediated by the transposase and any necessary accessory factors. Recombination in these cases may simply involve induction of a double-stranded break and strand transfer of a nearly intact duplex segment of DNA from donor to target (conservative transposition, Fig. 3a) or may involve fusion of the donor and target via duplication of the transposon (replicative transposition, Fig. 3B).

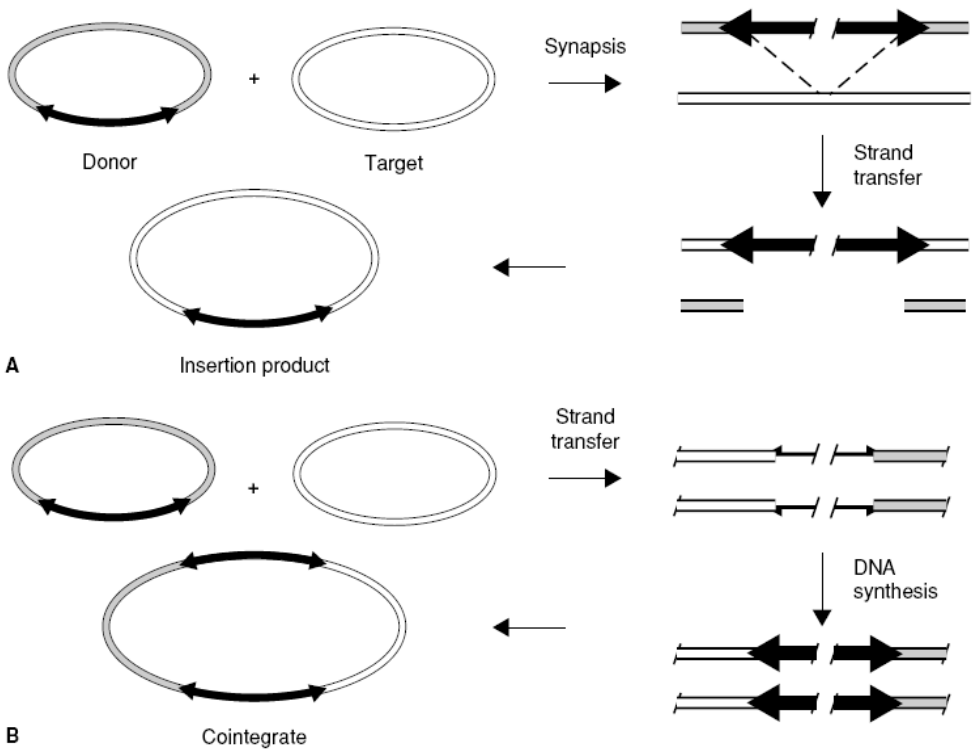


Fig. 3 Conservative and replicative transposition pathways. **A:** Conservative transposition. Strand transfer takes place between a nearly intact double-stranded DNA segment flanked by transposon ends. Integration into the target molecule leaves a double-stranded break in the donor molecule, which is lost during conservative transposition. **B:** Replicative transposition. Nicking occurs on the upper and lower DNA strands at opposite boundaries of the transposon; the free 3′ ends are transferred to the target DNA with the resulting single-stranded gaps being filled by DNA synthesis. Replicative transposition thereby generates a cointegrate product containing two copies of the transposable element.

The latter pathway is characterized by nicking of the upper and lower DNA strands at opposite boundaries of the transposon and transfer of both of the free 3^0 ends to the target DNA. This leads to duplicate copies of the transposon because the trans-poson/donor boundary remains intact on opposing ends of the element. The resulting single-stranded gap between duplex donor and target sequences, corresponding to the complementary strand of each copy of the transposon, is subsequently filled by DNA synthesis to generate a circular DNA molecule called a cointegrate. General aspects of these mechanisms apply to retroviral integration as well as mobile DNA elements; in the former case a complete DNA copy of the retroviral RNA is made by an element-en-coded reverse transcriptase and used as the donor in the transposition reaction.

In contrast, retrotransposition pathways involve participation of a reverse transcriptase activity directly in the recombination reaction. In these cases a DNA target site is cleaved and an exposed 3'-OH group in the cleaved DNA is used as a primer for reverse transcriptase, which uses a transposable-element RNA as the template. A complementary DNA segment is thereby copied directly from an RNA donor into the target site.

1. Phage Mu, a model transposable element

Upon infection of its E. coli host, phage Mu integrates by conservative transposition into the bacterial genome (see Fig. 3). The resulting prophage can be induced by a variety of environmental factors, resulting in multiple rounds of replicative transposition. In the Mu system, transposition is

obligatory for replication and thus the phage genome is replicated only as a cointegrate structure. Insertions occur at many sites in the genome, frequently within several kb of one another. In the final stage of the lytic cycle, packaging is initiated at one end of the Mu genome and continues until approximately 39 kb of DNA has been incorporated into the phage head. The wild-type Mu genome is 37.5 kb and thus about a 1.5 kb of host DNA adjacent to Mu is incorporated into the phage head. Deletion derivatives of Mu, called mini-Mu, can package still larger amounts of host DNA and are more useful as transducing phages.

The biochemistry of Mu transposition has been studied extensively. The transposase is a protein called MuA. MuA has both DNA binding and endonuclease activities needed to form the strand-transfer intermediate. However, MuA normally acts in a multiprotein complex with several accessory proteins (MuB, IHF, and HU). MuB is an activator of MuA and provides some degree of target-site selectivity, whereas HU is an E. coli non-sequence-specific DNA-binding protein. An extremely interesting feature of Mu is target immunity; copies of Mu do not insert into DNA molecules that already contain a copy of the transposon. This effect is achieved at the level of single DNA molecules and not through overall inhibition of transposition activity. The molecular basis for target immunity in the Mu system is the sequestration of MuB protein by copies of MuA that remain bound to MuA binding sites on the integrated transposon.

E. Illegitimate Recombination

Illegitimate recombination occurs at DNA sequences that share little or no homology with their exchange partners. These recombinases most likely comprise the most primitive of recombination systems because of their inherent lack of recognition specificity. Illegitimate recombination is divided into two classes; end-joining and strand-slippage. The end-joining reaction in eukaryotes is very efficient and has been shown to allow broken chromatids to undergo replication by fusing their ends together, presumably to prevent them from being recognized by DNA damage checkpoints and degraded. One of the hallmarks of end-joining in eukaryotes is that it readily occurs in the absence of homology. In prokaryotes, however, end-joining reactions do require short regions of micro-homology. Strand-slippage occurs most often in regions containing trior tetranucleotide repeats. These strand-slip-page reactions can delete or amplify these repetitive sequences often precipitating deleterious genetic defects.

Other illegitimate recombination reactions can be seen in type I and II topoisomerase reactions and in transposition or site-specific recombination events involving aberrant substrate molecules. Illegitimate recombination may also be an important player in the extensive genetic rearrangements that occur in cancerous cells. The loss of normal cell cycle checkpoints may allow damaged chromosomes to enter S phase and become subject to end-joining reactions or cause the accumulation of amplified palindromic sequences. Illegitimate recombination can mediate a number of chromosomal rearrangements, some of which may be deleterious to the organism, whereas others may lead to an evolutionarily favorable reorganization of the genome.