

CONCEPTS

The Punnett square is a shorthand method of predicting the genotypic and phenotypic ratios of progeny from a genetic cross.

✓ CONCEPT CHECK 4

If an F_1 plant depicted in Figure 3.5 is backcrossed to the parent with round seeds, what proportion of the progeny will have wrinkled seeds? (Use a Punnett square.)

- a. $\frac{3}{4}$ c. $\frac{1}{4}$
 b. $\frac{1}{2}$ d. 0

Probability as a tool in genetics Another method for determining the outcome of a genetic cross is to use the rules of probability, as Mendel did with his crosses. **Probability** expresses the likelihood of the occurrence of a particular event. It is the number of times that a particular event occurs, divided by the number of all possible outcomes. For example, a deck of 52 cards contains only one king of hearts. The probability of drawing one card from the deck at random and obtaining the king of hearts is $\frac{1}{52}$, because there is only one card that is the king of hearts (one event) and there are 52 cards that can be drawn from the deck (52 possible outcomes). The probability of drawing a card and obtaining an ace is $\frac{4}{52}$, because there are four cards that are aces (four events) and 52 cards (possible outcomes). Probability can be expressed either as a fraction ($\frac{4}{52}$ in this case) or as a decimal number (0.077 in this case).

The probability of a particular event may be determined by knowing something about *how* the event occurs or *how often* it occurs. We know, for example, that the probability of rolling a six-sided die and getting a four is $\frac{1}{6}$, because the die has six sides and any one side is equally likely to end up on top. So, in this case, understanding the nature of the event—the shape of the thrown die—allows us to determine the probability. In other cases, we determine the probability of an event by making a large number of observations. When a weather forecaster says that there is a 40% chance of rain on a particular day, this probability was obtained by observing a large number of days with similar atmospheric conditions and finding that it rains on 40% of those days. In this case, the probability has been determined empirically (by observation).

The multiplication rule Two rules of probability are useful for predicting the ratios of offspring produced in genetic crosses. The first is the **multiplication rule**, which states that the probability of two or more independent events occurring together is calculated by multiplying their independent probabilities.

To illustrate the use of the multiplication rule, let's again consider the roll of a die. The probability of rolling one die and obtaining a four is $\frac{1}{6}$. To calculate the probability of rolling a die twice and obtaining 2 fours, we can apply the multiplication rule. The probability of obtaining a four on the

(a) The multiplication rule

Roll 1

- 1 If you roll a die,...
- 2 ...in a large number of sample rolls, on average, one out of six times you will obtain a four;...
- 3 ...so the probability of obtaining a four in any roll is $\frac{1}{6}$.

Roll 2

- 4 If you roll the die again,...
- 5 ...your probability of getting four is again $\frac{1}{6}$;
- 6 ...so the probability of getting a four on two sequential rolls is $\frac{1}{6} \times \frac{1}{6} = \frac{1}{36}$.

(b) The addition rule

- 1 If you roll a die,...
- 2 ...on average, one out of six times you'll get a three...
- 3 ...and one out of six times you'll get a four.
- 4 That is, the probability of getting either a three or a four is $\frac{1}{6} + \frac{1}{6} = \frac{2}{6} = \frac{1}{3}$.

3.8 The multiplication and addition rules can be used to determine the probability of combinations of events.

first roll is $\frac{1}{6}$ and the probability of obtaining a four on the second roll is $\frac{1}{6}$; so the probability of rolling a four on both is $\frac{1}{6} \times \frac{1}{6} = \frac{1}{36}$ (**Figure 3.8a**). The key indicator for applying the multiplication rule is the word *and*; in the example just considered, we wanted to know the probability of obtaining a four on the first roll *and* a four on the second roll.

For the multiplication rule to be valid, the events whose joint probability is being calculated must be independent—the outcome of one event must not influence the outcome

of the other. For example, the number that comes up on one roll of the die has no influence on the number that comes up on the other roll; so these events are independent. However, if we wanted to know the probability of being hit on the head with a hammer and going to the hospital on the same day, we could not simply multiply the probability of being hit on the head with a hammer by the probability of going to the hospital. The multiplication rule cannot be applied here, because the two events are not independent—being hit on the head with a hammer certainly influences the probability of going to the hospital.

The addition rule The second rule of probability frequently used in genetics is the **addition rule**, which states that the probability of any one of two or more mutually exclusive events is calculated by adding the probabilities of these events. Let's look at this rule in concrete terms. To obtain the probability of throwing a die once and rolling *either* a three *or* a four, we would use the addition rule, adding the probability of obtaining a three ($\frac{1}{6}$) to the probability of obtaining a four (again, $\frac{1}{6}$), or $\frac{1}{6} + \frac{1}{6} = \frac{2}{6} = \frac{1}{3}$ (Figure 3.8b). The key indicators for applying the addition rule are the words *either* and *or*.

For the addition rule to be valid, the events whose probability is being calculated must be mutually exclusive, meaning that one event excludes the possibility of the occurrence of the other event. For example, you cannot throw a single die just once and obtain both a three and a four, because only one side of the die can be on top. These events are mutually exclusive.

CONCEPTS

The multiplication rule states that the probability of two or more independent events occurring together is calculated by multiplying their independent probabilities. The addition rule states that the probability that any one of two or more mutually exclusive events occurring is calculated by adding their probabilities.

✓ CONCEPT CHECK 5

If the probability of being blood-type A is $\frac{1}{8}$ and the probability of blood-type O is $\frac{1}{2}$, what is the probability of being either blood-type A or blood-type O?

- a. $\frac{5}{8}$ c. $\frac{1}{8}$
b. $\frac{1}{2}$ d. $\frac{1}{16}$

The application of probability to genetic crosses

The multiplication and addition rules of probability can be used in place of the Punnett square to predict the ratios of progeny expected from a genetic cross. Let's first consider a cross between two pea plants heterozygous for the locus that determines height, $Tt \times Tt$. Half of the gametes produced by each plant have a T allele, and the other half have a t allele; so the probability for each type of gamete is $\frac{1}{2}$.

The gametes from the two parents can combine in four different ways to produce offspring. Using the multiplication rule, we can determine the probability of each possible type. To calculate the probability of obtaining TT progeny, for example, we multiply the probability of receiving a T allele from the first parent ($\frac{1}{2}$) times the probability of receiving a T allele from the second parent ($\frac{1}{2}$). The multiplication rule should be used here because we need the probability of receiving a T allele from the first parent *and* a T allele from the second parent—two independent events. The four types of progeny from this cross and their associated probabilities are:

TT	(T gamete and T gamete)	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	tall
Tt	(T gamete and t gamete)	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	tall
tT	(t gamete and T gamete)	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	tall
tt	(t gamete and t gamete)	$\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$	short

Notice that there are two ways for heterozygous progeny to be produced: a heterozygote can either receive a T allele from the first parent and a t allele from the second or receive a t allele from the first parent and a T allele from the second.

After determining the probabilities of obtaining each type of progeny, we can use the addition rule to determine the overall phenotypic ratios. Because of dominance, a tall plant can have genotype TT , Tt , or tT ; so, using the addition rule, we find the probability of tall progeny to be $\frac{1}{4} + \frac{1}{4} + \frac{1}{4} = \frac{3}{4}$. Because only one genotype encodes short (tt), the probability of short progeny is simply $\frac{1}{4}$.

Two methods have now been introduced to solve genetic crosses: the Punnett square and the probability method. At this point, you may be asking, "Why bother with probability rules and calculations? The Punnett square is easier to understand and just as quick." For simple monohybrid crosses, the Punnett square is simpler than the probability method and is just as easy to use. However, for tackling more-complex crosses concerning genes at two or more loci, the probability method is both clearer and quicker than the Punnett square.

The binomial expansion and probability

When probability is used, it is important to recognize that there may be several different ways in which a set of events can occur. Consider two parents who are both heterozygous for albinism, a recessive condition in humans that causes reduced pigmentation in the skin, hair, and eyes (Figure 3.9; see also the introduction to Chapter 1). When two parents heterozygous for albinism mate ($Aa \times Aa$), the probability of their having a child with albinism (aa) is $\frac{1}{4}$ and the probability of having a child with normal pigmentation (AA or Aa) is $\frac{3}{4}$. Suppose we want to know the probability of this couple having three children, all three with albinism. In this case, there is only one way in which they can have three children with albinism: their first child has albinism *and* their second child has albinism *and* their third child has albinism. Here, we simply apply the multiplication rule: $\frac{1}{4} \times \frac{1}{4} \times \frac{1}{4} = \frac{1}{64}$.



3.9 Albinism in human beings is usually inherited as a recessive trait. [Richard Dranitzke/SS/Photo Researchers.]

Suppose we now ask, What is the probability of this couple having three children, one with albinism and two with normal pigmentation? This situation is more complicated. The first child might have albinism, whereas the second and third are unaffected; the probability of this sequence of events is $\frac{1}{4} \times \frac{3}{4} \times \frac{3}{4} = \frac{9}{64}$. Alternatively, the first and third child might have normal pigmentation, whereas the second has albinism; the probability of this sequence is $\frac{3}{4} \times \frac{1}{4} \times \frac{3}{4} = \frac{9}{64}$. Finally, the first two children might have normal pigmentation and the third albinism; the probability of this sequence is $\frac{3}{4} \times \frac{3}{4} \times \frac{1}{4} = \frac{9}{64}$. Because *either* the first sequence *or* the second sequence *or* the third sequence produces one child with albinism and two with normal pigmentation, we apply the addition rule and add the probabilities: $\frac{9}{64} + \frac{9}{64} + \frac{9}{64} = \frac{27}{64}$.

If we want to know the probability of this couple having five children, two with albinism and three with normal pigmentation, figuring out *all* the different combinations of children and their probabilities becomes more difficult. This task is made easier if we apply the binomial expansion.

The binomial takes the form $(p + q)^n$, where p equals the probability of one event, q equals the probability of the alternative event, and n equals the number of times the event occurs. For figuring the probability of two out of five children with albinism:

p = the probability of a child having albinism ($\frac{1}{4}$)

q = the probability of a child having normal pigmentation ($\frac{3}{4}$)

The binomial for this situation is $(p + q)^5$ because there are five children in the family ($n = 5$). The expansion is:

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

Each of the terms in the expansion provides the probability for one particular combination of traits in the children. The first term in the expansion (p^5) equals the probability of having five children all with albinism, because p is the probability of albinism. The second term ($5p^4q$) equals the probability of having four children with albinism and one with normal pigmentation, the third term ($10p^3q^2$) equals the probability of having three children with albinism and two with normal pigmentation, and so forth.

To obtain the probability of any combination of events, we insert the values of p and q ; so the probability of having two out of five children with albinism is:

$$10p^2q^3 = 10\left(\frac{1}{4}\right)^2\left(\frac{3}{4}\right)^3 = \frac{270}{1024} = 0.26$$

We could easily figure out the probability of any desired combination of albinism and pigmentation among five children by using the other terms in the expansion.

How did we expand the binomial in this example? In general, the expansion of any binomial $(p + q)^n$ consists of a series of $n + 1$ terms. In the preceding example, $n = 5$; so there are $5 + 1 = 6$ terms: p^5 , $5p^4q$, $10p^3q^2$, $10p^2q^3$, $5pq^4$, and q^5 . To write out the terms, first figure out their exponents. The exponent of p in the first term always begins with the power to which the binomial is raised, or n . In our example, n equals 5, so our first term is p^5 . The exponent of p decreases by one in each successive term; so the exponent of p is 4 in the second term (p^4), 3 in the third term (p^3), and so forth. The exponent of q is 0 (no q) in the first term and increases by 1 in each successive term, increasing from 0 to 5 in our example.

Next, determine the coefficient of each term. The coefficient of the first term is always 1; so, in our example, the first term is $1p^5$, or just p^5 . The coefficient of the second term is always the same as the power to which the binomial is raised; in our example, this coefficient is 5 and the term is $5p^4q$. For the coefficient of the third term, look back at the preceding term; multiply the coefficient of the preceding term (5 in our example) by the exponent of p in that term (4) and then divide by the number of that term (second term, or 2). So the coefficient of the third term in our example is $(5 \times 4)/2 = 20/2 = 10$ and the term is $10p^3q^2$. Follow this procedure for each successive term.

Another way to determine the probability of any particular combination of events isn't to use the following formula:

$$P = \frac{n!}{s!t!} p^s q^t$$

where P equals the overall probability of event X with probability p occurring s times and event Y with probability q occurring t times. For our albinism example, event X would be the occurrence of a child with albinism ($\frac{1}{4}$) and event Y would be the occurrence of a child with normal pigmentation ($\frac{3}{4}$); s would equal the number of children with albi-

nism (2) and t would equal the number of children with normal pigmentation (3). The ! symbol stands for factorial, and it means the product of all the integers from n to 1. In this example, $n = 5$; so $n! = 5 \times 4 \times 3 \times 2 \times 1$. Applying this formula to obtain the probability of two out of five children having albinism, we obtain:

$$P = \frac{5!}{2!3!} \left(\frac{1}{4}\right)^2 \left(\frac{3}{4}\right)^3$$

$$= \frac{5 \times 4 \times 3 \times 2 \times 1}{2 \times 1 \times 3 \times 2 \times 1} \left(\frac{1}{4}\right)^2 \left(\frac{3}{4}\right)^3 = 0.26$$

This value is the same as that obtained with the binomial expansion. **TRY PROBLEMS 23, 24, AND 25** →

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 3.7 illustrates a testcross (in this case, it is also 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 ($TT \times tt \rightarrow$ all Tt); if the plant were heterozygous (Tt), half of the progeny would be tall and half would be short ($Tt \times tt \rightarrow \frac{1}{2} Tt$ and $\frac{1}{2} tt$). 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.

TRY PROBLEMS 17 AND 19 →

CONCEPTS

The binomial expansion can be used to determine the probability of a particular set of events. A testcross is a cross between an individual with an unknown genotype and one with a homozygous recessive genotype. The outcome of the testcross can reveal the unknown genotype.

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 common allele for a character—called the **wild type** because it is the allele usually found in the wild—is

often symbolized by one or more letters and a plus sign (+). The letter or letters chosen are usually based on the mutant (unusual) phenotype. 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. In another way of distinguishing alleles, the first letter is lowercase if the mutant phenotype is recessive and it is uppercase if the mutant phenotype is dominant: for example, narrow leaflet (ln) in soybeans is recessive to broad leaflet (Ln). Superscripts and subscripts are sometimes added to distinguish between genes: Lfr_1 and Lfr_2 represent dominant mutant 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. For example, the genotype of a goat that is heterozygous for restricted ears might be written El^+/El^R or simply $+/El^R$. If genotypes at more than one locus are presented together, a space separates the genotypes. For example, a goat heterozygous for a pair of alleles that produces restricted ears and heterozygous for another pair of alleles that produces goiter can be designated by $El^+/El^R G/g$.

CONNECTING CONCEPTS

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 and one of the alleles is dominant over the other. 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 three phenotypic ratios to understand (**Table 3.3**). The 3 : 1 ratio arises in a simple genetic cross when both of the

Table 3.3 Phenotypic ratios for simple genetic crosses (crosses for a single locus) with dominance

Phenotypic Ratio	Genotypes of Parents	Genotypes of Progeny
3 : 1	$Aa \times Aa$	$\frac{3}{4} A_ : \frac{1}{4} aa$
1 : 1	$Aa \times aa$	$\frac{1}{2} Aa : \frac{1}{2} aa$
Uniform progeny	$AA \times AA$ $aa \times aa$ $AA \times aa$ $AA \times Aa$	All AA All aa All Aa All $A_$

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

Table 3.4 Genotypic ratios for simple genetic crosses (crosses for a single locus)

Genotypic Ratio	Genotypes of Parents	Genotypes of Progeny
1 : 2 : 1	$Aa \times Aa$	$\frac{1}{4} AA : \frac{1}{2} Aa : \frac{1}{4} aa$
1 : 1	$Aa \times aa$ $Aa \times AA$	$\frac{1}{2} Aa : \frac{1}{2} aa$ $\frac{1}{2} Aa : \frac{1}{2} AA$
Uniform progeny	$AA \times AA$ $aa \times aa$ $AA \times aa$	All AA All aa All Aa

parents are heterozygous for a dominant trait ($Aa \times Aa$). The second phenotypic ratio is the 1 : 1 ratio, which results from the mating of a homozygous parent and a heterozygous parent. The homozygous parent in this cross must carry two recessive alleles ($Aa \times aa$) to obtain a 1 : 1 ratio, because a cross between a homozygous dominant parent and a heterozygous parent ($AA \times Aa$) produces offspring displaying only the dominant trait.

The third phenotypic ratio is not really a ratio: all the offspring have the same phenotype (uniform progeny). Several combinations of parents can produce this outcome (see Table 3.3). A cross between any two homozygous parents—either between two of the same homozygotes ($AA \times AA$ and $aa \times aa$) or between two different homozygotes ($AA \times aa$)—produces progeny all having the same phenotype. Progeny of a single phenotype also can result from a cross between a homozygous dominant parent and a heterozygote ($AA \times Aa$).

If we are interested in the ratios of genotypes instead of phenotypes, there are only three outcomes to remember (Table 3.4): 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. 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.

3.3 Dihybrid Crosses Reveal the Principle of Independent Assortment

We will now extend Mendel's principle of segregation to more-complex crosses entailing 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 crossed varieties of peas that differed in *two* characteristics—a **dihybrid cross**. For example, he had one homozygous variety of pea with seeds that were round and yellow;

another homozygous variety with seeds that were wrinkled and green. When he crossed the two varieties, the seeds of all the F_1 progeny were round and yellow. He then self-fertilized the F_1 and obtained the following progeny in the F_2 : 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, $\frac{9}{16}$ of the progeny were round and yellow, $\frac{3}{16}$ were wrinkled and yellow, $\frac{3}{16}$ were round and green, and $\frac{1}{16}$ 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 F_2 . 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 (see Table 3.2).

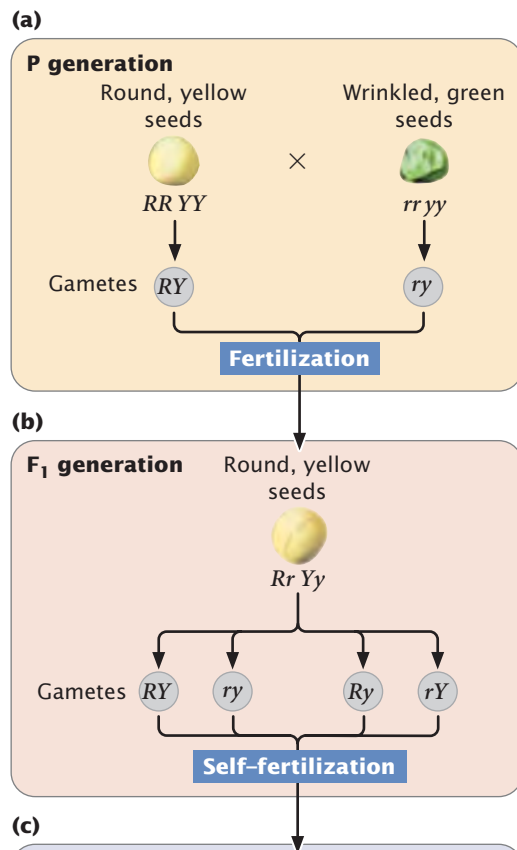
A common mistake is to think that the principle of segregation and the principle of independent assortment refer to two different processes. The principle of independent assortment is really an extension of the principle of segregation. The principle of segregation states that the two alleles of a locus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

Let's see how the principle of independent assortment explains the results that Mendel obtained in his dihybrid cross. Each plant possesses two alleles encoding each characteristic, and so the parental plants must have had genotypes $RR YY$ and $rr yy$ (Figure 3.10a). 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 F_1 , all with genotype $Rr Yy$. Because round is dominant over wrinkled and yellow is dominant over green, the phenotype of the F_1 will be round and yellow.

When Mendel self-fertilized the F_1 plants to produce the F_2 , the alleles for each locus separated, with one allele going into each gamete. This event 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 3.10b). When these four types of gametes are combined to produce the F_2 generation, the progeny con-

Experiment

Question: Do alleles encoding different traits separate independently?

Methods**Results**

Phenotypic ratio

9 round, yellow : 3 round, green :
3 wrinkled, yellow : 1 wrinkled, green

Conclusion: The allele encoding color separated independently of the allele encoding seed shape, producing a 9 : 3 : 3 : 1 ratio in the F₂ progeny.

3.10 Mendel's dihybrid crosses revealed the principle of independent assortment.

www.whfreeman.com/pierce4e Set up your own crosses and explore Mendel's principles of heredity in Animation 3.1.

sist of $\frac{9}{16}$ round and yellow, $\frac{3}{16}$ wrinkled and yellow, $\frac{3}{16}$ round and green, and $\frac{1}{16}$ wrinkled and green, resulting in a 9 : 3 : 3 : 1 phenotypic ratio (Figure 3.10c).

Relating 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 in meiosis. Each pair of homologous chromosomes separates independently of all other pairs in anaphase I of meiosis (see Figure 2.17); 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, as will be discussed fully in Chapter 7).

CONCEPTS

The principle of independent assortment states that genes encoding different characteristics separate independently of one another when gametes are formed, owing to the independent separation of homologous pairs of chromosomes in meiosis. Genes located close together on the same chromosome do not, however, assort independently.

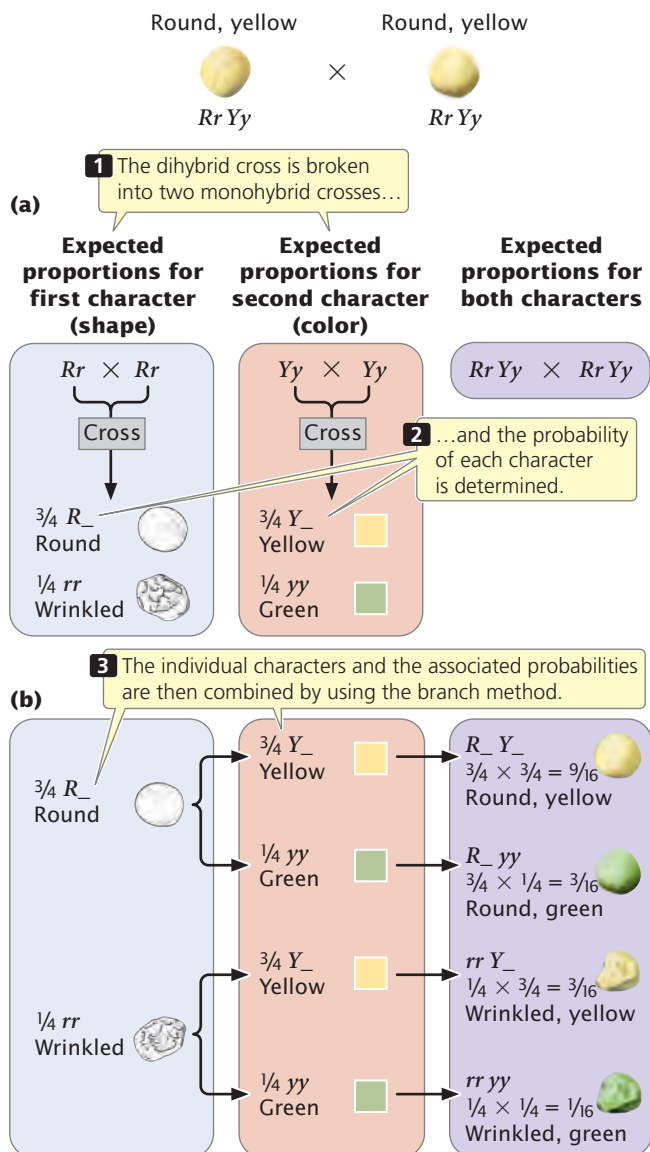
✓ **CONCEPT CHECK 6**

How are the principles of segregation and independent assortment related and how are they different?

Applying Probability and the Branch Diagram to Dihybrid Crosses

When the genes at two loci separate independently, a dihybrid cross can be understood as two monohybrid crosses. Let's examine Mendel's dihybrid cross ($Rr Yy \times Rr Yy$) by considering each characteristic separately (Figure 3.11a). If we consider only the shape of the seeds, the cross was $Rr \times Rr$, which yields a 3 : 1 phenotypic ratio ($\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled progeny, see Table 3.3). Next consider the other characteristic, the color of the seed. The cross was $Yy \times Yy$, which produces a 3 : 1 phenotypic ratio ($\frac{3}{4}$ yellow and $\frac{1}{4}$ green progeny).

We can now combine these monohybrid ratios by using the multiplication rule to obtain the proportion of progeny with different combinations of seed shape and color. The proportion of progeny with round and yellow seeds is $\frac{3}{4}$ (the probability of round) \times $\frac{3}{4}$ (the probability of yellow) = $\frac{9}{16}$. The proportion of progeny with round and



3.11 A branch diagram can be used to determine the phenotypes and expected proportions of offspring from a dihybrid cross ($Rr Yy \times Rr Yy$).

green seeds is $\frac{3}{4} \times \frac{1}{4} = \frac{3}{16}$; the proportion of progeny with wrinkled and yellow seeds is $\frac{1}{4} \times \frac{3}{4} = \frac{3}{16}$; and the proportion of progeny with wrinkled and green seeds is $\frac{1}{4} \times \frac{1}{4} = \frac{1}{16}$.

Branch diagrams are a convenient way of organizing all the combinations of characteristics (Figure 3.11b). In the first column, list the proportions of the phenotypes for one character (here, $\frac{3}{4}$ round and $\frac{1}{4}$ wrinkled). In the second column, list the proportions of the phenotypes for the second character ($\frac{3}{4}$ yellow and $\frac{1}{4}$ green) twice, next to each of the phenotypes in the first column: put $\frac{3}{4}$ yellow and $\frac{1}{4}$ green next to the round phenotype and again next to the wrinkled phenotype. Draw lines between the phenotypes in the first column and each of the phenotypes in the second

column. Now follow each branch of the diagram, multiplying the probabilities for each trait along that branch. One branch leads from round to yellow, yielding round and yellow progeny. Another branch leads from round to green, yielding round and green progeny, and so forth. We calculate the probability of progeny with a particular combination of traits by using the multiplication rule: the probability of round ($\frac{3}{4}$) and yellow ($\frac{3}{4}$) seeds is $\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$. The advantage of the branch diagram is that it helps keep track of all the potential combinations of traits that may appear in the progeny. It can be used to determine phenotypic or genotypic ratios for any number of characteristics.

Using probability is much faster than using the Punnett square for crosses that include multiple loci. Genotypic and phenotypic ratios can be quickly worked out by combining, with the multiplication rule, the simple ratios in Tables 3.3 and 3.4. The probability method is particularly efficient if we need the probability of only a particular phenotype or genotype among the progeny of a cross. Suppose we needed to know the probability of obtaining the genotype $Rr yy$ in the F_2 of the dihybrid cross in Figure 3.10. The probability of obtaining the Rr genotype in a cross of $Rr \times Rr$ is $\frac{1}{2}$ and that of obtaining yy progeny in a cross of $Yy \times Yy$ is $\frac{1}{4}$ (see Table 3.4). Using the multiplication rule, we find the probability of $Rr yy$ to be $\frac{1}{2} \times \frac{1}{4} = \frac{1}{8}$.

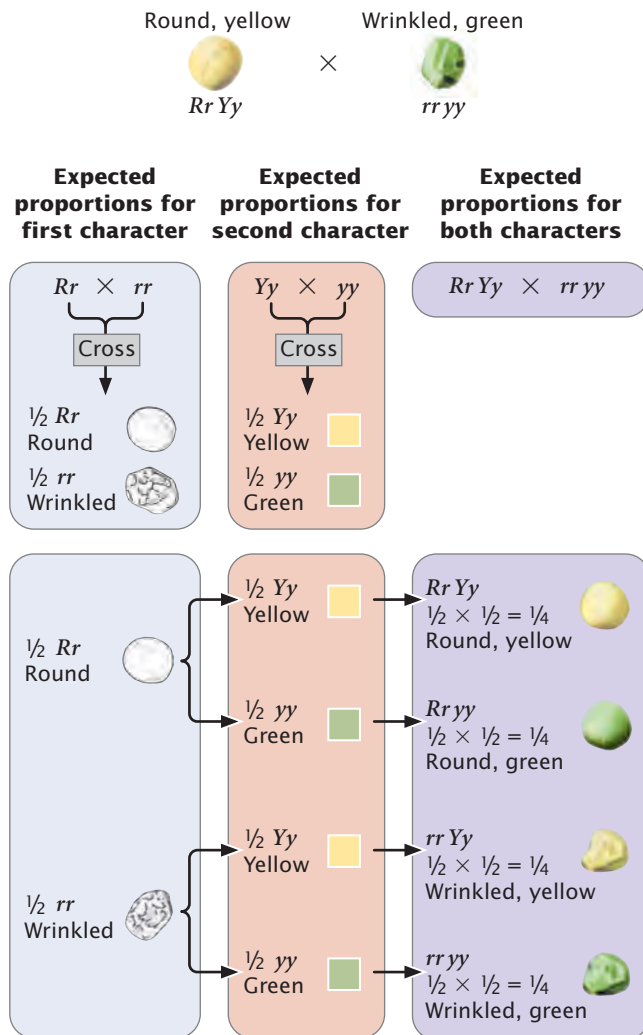
To illustrate the advantage of the probability method, consider the cross $Aa Bb cc Dd Ee \times Aa Bb Cc dd Ee$. Suppose we wanted to know the probability of obtaining offspring with the genotype $aa bb cc dd ee$. If we used a Punnett square to determine this probability, we might be working on the solution for months. However, we can quickly figure the probability of obtaining this one genotype by breaking this cross into a series of single-locus crosses:

Progeny cross	Genotype	Probability
$Aa \times Aa$	aa	$\frac{1}{4}$
$Bb \times Bb$	bb	$\frac{1}{4}$
$cc \times Cc$	cc	$\frac{1}{2}$
$Dd \times dd$	dd	$\frac{1}{2}$
$Ee \times Ee$	ee	$\frac{1}{4}$

The probability of an offspring from this cross having genotype $aa bb cc dd ee$ is now easily obtained by using the multiplication rule: $\frac{1}{4} \times \frac{1}{4} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{4} = \frac{1}{256}$. This calculation assumes that genes at these five loci all assort independently.

CONCEPTS

A cross including several characteristics can be worked by breaking the cross down into single-locus crosses and using the multiplication rule to determine the proportions of combinations of characteristics (provided that the genes assort independently).



3.12 A branch diagram can be used to determine the phenotypes and expected proportions of offspring from a dihybrid testcross ($Rr Yy \times rr yy$).

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 F_1 plants ($Rr Yy$) obtained by Mendel in his dihybrid cross and the wrinkled and green plants ($rr yy$), as depicted in **Figure 3.12**. Break the cross down into a series of single-locus crosses. The cross $Rr \times rr$ yields $\frac{1}{2}$ round (Rr) progeny and $\frac{1}{2}$ wrinkled (rr) progeny. The cross $Yy \times yy$ yields $\frac{1}{2}$ yellow (Yy) progeny and $\frac{1}{2}$ green (yy) progeny. Using the multiplication rule, we find the proportion of round and yellow progeny to be $\frac{1}{2}$ (the probability of round) \times $\frac{1}{2}$ (the probability of yellow) = $\frac{1}{4}$. Four combinations of traits with the following proportions appear in the offspring: $\frac{1}{4}$ $Rr Yy$, round yellow; $\frac{1}{4}$ $Rr yy$, round green; $\frac{1}{4}$ $rr Yy$, wrinkled yellow; and $\frac{1}{4}$ $rr yy$, wrinkled green.

Worked Problem

Not only are the principles of segregation and independent assortment important because they explain how heredity works, but they also provide the means for predicting the outcome of genetic crosses. This predictive power has made genetics a powerful tool in agriculture and other fields, and the ability to apply the principles of heredity is an important skill for all students of genetics. Practice with genetic problems is essential for mastering the basic principles of heredity; no amount of reading and memorization can substitute for the experience gained by deriving solutions to specific problems in genetics.

Students may have difficulty with genetics problems when they are unsure of where to begin or how to organize the problem and plan a solution. In genetics, every problem is different, and so no common series of steps can be applied to all genetics problems. Logic and common sense must be used to analyze a problem and arrive at a solution. Nevertheless, certain steps can facilitate the process, and solving the following problem will serve to illustrate these steps.

In mice, black coat color (B) is dominant over brown (b), and a solid pattern (S) is dominant over white spotted (s). Color and spotting are controlled by genes that assort independently. A homozygous black, spotted mouse is crossed with a homozygous brown, solid mouse. All the F_1 mice are black and solid. A testcross is then carried out by mating the F_1 mice with brown, spotted mice.

- Give the genotypes of the parents and the F_1 mice.
- Give the genotypes and phenotypes, along with their expected ratios, of the progeny expected from the testcross.

• Solution

Step 1. Determine the questions to be answered. What question or questions is the problem asking? Is it asking for genotypes, genotypic ratios, or phenotypic ratios? This problem asks you to provide the *genotypes* of the parents and the F_1 , the *expected genotypes* and *phenotypes* of the progeny of the testcross, and their *expected proportions*.

Step 2. Write down the basic information given in the problem. This problem provides important information about the dominance relations of the characters and about the mice being crossed. Black is dominant over brown, and solid is dominant over white spotted. Furthermore, the genes for the two characters assort independently. In this problem, symbols are provided for the different alleles (B for black, b for brown, S for solid, and s for spotted); had these symbols not been provided, you would need to choose symbols to represent these alleles.

bb *SS* in the P generation. Do these genotypes encode the phenotypes given in the problem? Are the F₁ progeny phenotypes consistent with the genotypes that you assigned? The answers are consistent with the information.

➔ Now that we have stepped through a genetics problem together, try your hand at Problem 30 at the end of the chapter.

3.4 Observed Ratios of Progeny May Deviate from Expected Ratios by Chance

When two individual organisms 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 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— $\frac{1}{2}$ heads and $\frac{1}{2}$ tails. If you flip a coin 1000 times, the proportion of heads and tails obtained will probably be very close to that expected 1 : 1 ratio. However, if you flipped 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 heads and 7 tails, just by chance. 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.

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. This hypothesis, that chance alone is responsible for any deviations between observed and expected values, is sometimes called the null hypothesis. When the probability calculated from the chi-square test is high, we assume that chance alone produced the difference (the null hypothesis is true). When the probability is low, we assume that some factor other than chance—some significant factor—produced the deviation (the null hypothesis is false).

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* × *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 $\frac{3}{4} \times 50 = 37.5$ and the expected number of gray kittens is $\frac{1}{4} \times 50 = 12.5$. The chi-square (χ^2) value is calculated by using the following formula:

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

Table 3.5 Critical values of the χ^2 distribution

df	P								
	0.995	0.975	0.9	0.5	0.1	0.05*	0.025	0.01	0.005
1	0.000	0.000	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.

*Most scientists assume that, when $P < 0.05$, a significant difference exists between the observed and the expected values in a chi-square test.

where Σ means the sum. We calculate the sum of all the squared differences between observed and expected and divide by the expected values. To calculate the chi-square value for our black and gray kittens, we 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 $(\text{observed} - \text{expected})^2/\text{expected}$ 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 expected 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 3.5**). 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 0.025, and the value of 6.635, associated with a probability of 0.01.

Thus, the probability associated with our chi-square value is less than 0.025 and greater than 0.01. 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 0.05 probability level as their cutoff value: if the probability of chance being responsible for the deviation is greater than or equal to 0.05, they accept that chance may be responsible for the deviation between the observed and the expected values. When the probability is less than 0.05, 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 had a greater mortality rate before the progeny were counted or perhaps other genetic factors skewed the observed ratios.

In choosing 0.05 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. Bear in mind that, even if we obtain a probability of, say, 0.01, there is still a 1% probability that the deviation between the observed and the expected numbers is due to nothing more than chance. Calculation of the chi-square value is illustrated in **Figure 3.13**. **TRY PROBLEM 35** →

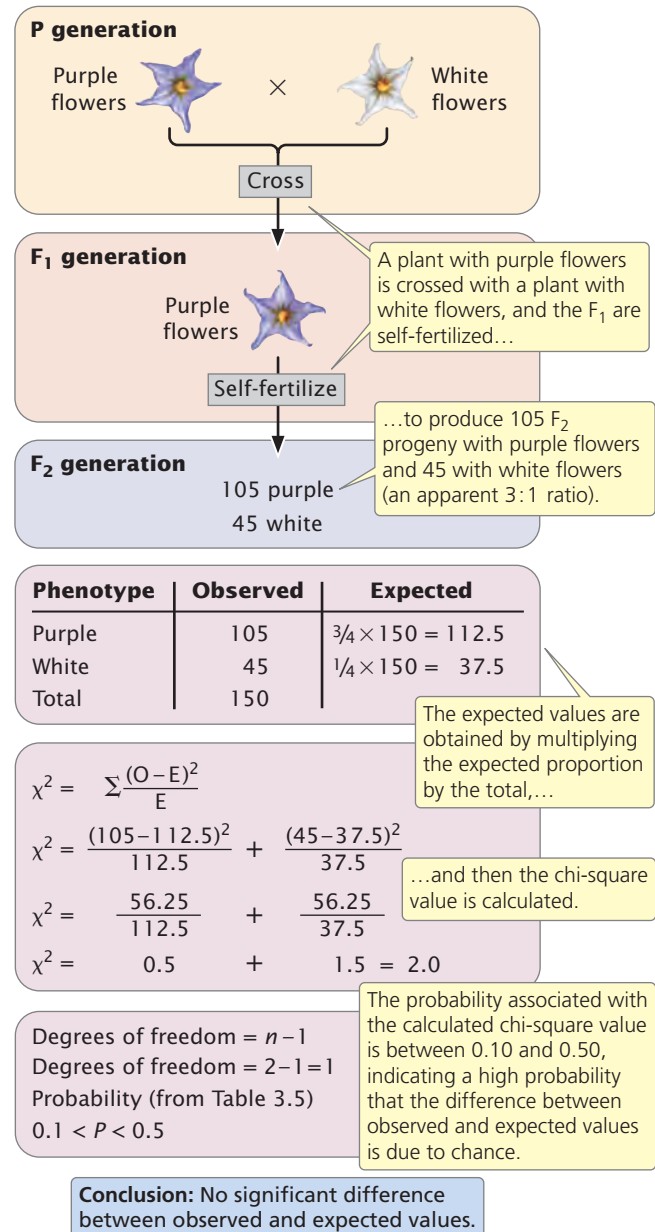
CONCEPTS

Differences between observed and expected ratios can arise by chance. The goodness-of-fit chi-square test can be used to evaluate whether deviations between observed and expected numbers are likely to be due to chance or to some other significant factor.

✓ CONCEPT CHECK 7

A chi-square test comparing observed and expected progeny is carried out, and the probability associated with the calculated chi-square value is 0.72. What does this probability represent?

- Probability that the correct results were obtained
- Probability of obtaining the observed numbers
- Probability that the difference between observed and expected numbers is significant
- Probability that the difference between observed and expected numbers could be due to chance



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

CONCEPTS SUMMARY

- Gregor Mendel discovered the principles of heredity. His success can be attributed to his choice of the pea plant as an experimental organism, the use of characters with a few easily distinguishable phenotypes, his experimental approach, the use of mathematics to interpret his results, and careful attention to detail.
- Genes are inherited factors that determine a characteristic. Alternate forms of a gene are called alleles. The alleles are located at a specific place, a locus, on a chromosome, and the

set of genes that an individual organism possesses is its genotype. Phenotype is the manifestation or appearance of a characteristic and may refer to a physical, biochemical, or behavioral characteristic. Only the genotype—not the phenotype—is inherited.

- The principle of segregation states that an individual organism possesses two alleles encoding a trait and that these two alleles separate in equal proportions when gametes are formed.