

# Answers to All Questions and Problems

## CHAPTER 1

**1.1** In a few sentences, what were Mendel's key ideas about inheritance?

**ANS:** Mendel postulated transmissible factors—genes—to explain the inheritance of traits. He discovered that genes exist in different forms, which we now call alleles. Each organism carries two copies of each gene. During reproduction, one of the gene copies is randomly incorporated into each gamete. When the male and female gametes unite at fertilization, the gene copy number is restored to two. Different alleles may coexist in an organism. During the production of gametes, they separate from each other without having been altered by coexistence.

**1.2** Both DNA and RNA are composed of nucleotides. What molecules combine to form a nucleotide?

**ANS:** Each nucleotide consists of a sugar, a nitrogen-containing base, and a phosphate.

**1.3** Which bases are present in DNA? Which bases are present in RNA? Which sugars are present in each of these nucleic acids?

**ANS:** The bases present in DNA are adenine, thymine, guanine, and cytosine; the bases present in RNA are adenine, uracil, guanine, and cytosine. The sugar in DNA is deoxyribose; the sugar in RNA is ribose.

**1.4** What is a genome?

**ANS:** A genome is the set of all the DNA molecules that are characteristic of an organism. Each DNA molecule forms one chromosome in a cell of the organism.

**1.5** The sequence of a strand of DNA is ATTGCCGTC. If this strand serves as the template for DNA synthesis, what will be the sequence of the newly synthesized strand?

**ANS:** TAACGGCAG

**1.6** A gene contains 141 codons. How many nucleotides are present in the gene's coding sequence? How many amino acids are expected to be present in the polypeptide encoded by this gene?

**ANS:** There are  $3 \times 141 = 423$  nucleotides in the gene's coding sequence. Its polypeptide product will contain 141 amino acids.

**1.7** The template strand of a gene being transcribed is CTT-GCCAGT. What will be the sequence of the RNA made from this template?

**ANS:** GAACGGUCT

**1.8** What is the difference between transcription and translation?

**ANS:** Transcription is the production of an RNA chain using a DNA chain as a template. Translation is the production of a chain of amino acids—that is, a polypeptide—using an RNA chain as a template.

**1.9** RNA is synthesized using DNA as a template. Is DNA ever synthesized using RNA as a template? Explain.

**ANS:** Sometimes, DNA is synthesized from RNA in a process called reverse transcription. This process plays an important role in the life cycles of some viruses.

**1.10** The gene for  $\alpha$ -globin is present in all vertebrate species. Over millions of years, the DNA sequence of this gene has changed in the lineage of each species. Consequently, the amino acid sequence of  $\alpha$ -globin has also changed in these lineages. Among the 141 amino acid positions in this polypeptide, human  $\alpha$ -globin differs from shark  $\alpha$ -globin in 79 positions; it differs from carp  $\alpha$ -globin in 68 and from cow  $\alpha$ -globin in 17. Do these data suggest an evolutionary phylogeny for these vertebrate species?

**ANS:** The human and cow  $\alpha$ -globins are least different; therefore, on the assumption that differences in  $\alpha$ -globin reflect the degree of phylogenetic relationship, the human and the cow are the most closely related organisms among those mentioned. The next closest "relative" of humans is the carp, and the most distant relative is the shark.

**1.11** Sickle-cell anemia is caused by a mutation in one of the codons in the gene for  $\beta$ -globin; because of this mutation, the sixth amino acid in the  $\beta$ -globin polypeptide is a valine instead of a glutamic acid. A less severe type of anemia is caused by a mutation that changes this same codon

to one specifying lysine as the sixth amino acid in the  $\beta$ -globin polypeptide. What word is used to describe the two mutant forms of this gene? Do you think that an individual carrying these two mutant forms of the  $\beta$ -globin gene would suffer from anemia? Explain.

**ANS:** The two mutant forms of the  $\beta$ -globin gene are properly described as alleles. Because neither of the mutant alleles can specify a “normal” polypeptide, an individual who carries each of them would probably suffer from anemia.

**1.12** Hemophilia is an inherited disorder in which the blood-clotting mechanism is defective. Because of this defect, people with hemophilia may die from cuts or bruises, especially if internal organs such as the liver, lungs, or kidneys have been damaged. One method of treatment involves injecting a blood-clotting factor that has been purified from blood donations. This factor is a protein encoded by a human gene. Suggest a way in which modern genetic technology could be used to produce this factor on an industrial scale. Is there a way in which the inborn error of hemophilia could be corrected by human gene therapy?

**ANS:** The gene for the human clotting factor could be isolated from the human genome and transferred into bacteria, which could then be grown in vats to produce large amounts of the gene’s protein product. This product could be isolated from the bacteria, purified, and then injected into patients to treat hemophilia. Another approach would be to transfer a normal copy of the clotting factor gene into the cells of people who have hemophilia. If expressed properly, the transferred normal gene might be able to compensate for the mutant allele these people naturally carry. For this approach to succeed, the normal clotting factor gene would have to be transferred into the cells that produce clotting factor, or into their precursors.

## CHAPTER 2

**2.1** Carbohydrates and proteins are linear polymers. What types of molecules combine to form these polymers?

**ANS:** Sugars combine to form carbohydrates; amino acids combine to form proteins.

**2.2** All cells are surrounded by a membrane; some cells are surrounded by a wall. What are the differences between cell membranes and cell walls?

**ANS:** Cell membranes are made of lipids and proteins; they have a fluid structure. Cell walls are made of more rigid materials such as cellulose.

**2.3** What are the principal differences between prokaryotic and eukaryotic cells?

**ANS:** In a eukaryotic cell, the many chromosomes are contained within a membrane-bounded structure called the nucleus; the chromosomes of prokaryotic cells are not contained within a special subcellular compartment.

Eukaryotic cells usually possess a well-developed internal system of membranes and they also have membrane-bounded subcellular organelles such as mitochondria and chloroplasts; prokaryotic cells do not typically have a system of internal membranes (although some do), nor do they possess membrane-bounded organelles.

**2.4** Distinguish between the haploid and diploid states. What types of cells are haploid? What types of cells are diploid?

**ANS:** In the haploid state, each chromosome is represented once; in the diploid state, each chromosome is represented twice. Among multicellular eukaryotes, gametes are haploid and somatic cells are diploid.

**2.5** Compare the sizes and structures of prokaryotic and eukaryotic chromosomes.

**ANS:** Prokaryotic chromosomes are typically (but not always) smaller than eukaryotic chromosomes; in addition, prokaryotic chromosomes are circular, whereas eukaryotic chromosomes are linear. For example, the circular chromosome of *E. coli*, a prokaryote, is about 1.4 mm in circumference. By contrast, a linear human chromosome may be 10–30 cm long. Prokaryotic chromosomes also have a comparatively simple composition: DNA, some RNA, and some protein. Eukaryotic chromosomes are more complex: DNA, some RNA, and a lot of protein.

**2.6** With a focus on the chromosomes, what are the key events during interphase and M phase in the eukaryotic cell cycle?

**ANS:** During interphase, the chromosomes duplicate. During M phase (mitosis), the duplicated chromosomes, each consisting of two identical sister chromatids, condense (a feature of prophase), migrate to the equatorial plane of the cell (a feature of metaphase), and then split so that their constituent sister chromatids are separated into different daughter cells (a feature of anaphase); this last process is called sister chromatid disjunction.

**2.7** Which typically lasts longer, interphase or M phase? Can you explain why one of these phases lasts longer than the other?

**ANS:** Interphase typically lasts longer than M phase. During interphase, DNA must be synthesized to replicate all the chromosomes. Other materials must also be synthesized to prepare for the upcoming cell division.

**2.8** In what way do the microtubule organizing centers of plant and animal cells differ?

**ANS:** The microtubule organizing centers of animal cells have distinct centrosomes, whereas the microtubule organizing centers of plant cells do not.

**2.9** Match the stages of mitosis with the events they encompass: Stages: (1) anaphase, (2) metaphase, (3) prophase, and (4) telophase. Events: (a) reformation of the nucleolus, (b) disappearance of the nuclear membrane,

(c) condensation of the chromosomes, (d) formation of the mitotic spindle, (e) movement of chromosomes to the equatorial plane, (f) movement of chromosomes to the poles, (g) decondensation of the chromosomes, (h) splitting of the centromere, and (i) attachment of microtubules to the kinetochore.

**ANS:** (1) Anaphase: (f), (h); (2) metaphase: (e), (i); (3) prophase: (b), (c), (d); (4) telophase: (a), (g).

**2.10** Arrange the following events in the correct temporal sequence during eukaryotic cell division, starting with the earliest: (a) condensation of the chromosomes, (b) movement of chromosomes to the poles, (c) duplication of the chromosomes, (d) formation of the nuclear membrane, (e) attachment of microtubules to the kinetochores, and (f) migration of centrosomes to positions on opposite sides of the nucleus.

**ANS:** (c), (f), (a), (e), (b), (d).

**2.11** In human beings, the gene for  $\beta$ -globin is located on chromosome 11, and the gene for  $\alpha$ -globin, which is another component of the hemoglobin protein, is located on chromosome 16. Would these two chromosomes be expected to pair with each other during meiosis? Explain your answer.

**ANS:** Chromosomes 11 and 16 would not be expected to pair with each other during meiosis; these chromosomes are heterologues, not homologues.

**2.12** A sperm cell from the fruit fly *Drosophila melanogaster* contains four chromosomes. How many chromosomes would be present in a spermatogonial cell about to enter meiosis? How many chromatids would be present in a spermatogonial cell at metaphase I of meiosis? How many would be present at metaphase II?

**ANS:** There are eight chromosomes in a *Drosophila* spermatogonial cell about to enter meiosis. There are 16 chromatids in a *Drosophila* spermatogonial cell at metaphase I of meiosis. There are eight chromatids in a *Drosophila* cell at metaphase II of meiosis.

**2.13** Does crossing over occur before or after chromosome duplication in cells going through meiosis?

**ANS:** Crossing over occurs *after* chromosomes have duplicated in cells going through meiosis.

**2.14** What visible characteristics of chromosomes indicate that they have undergone crossing over during meiosis?

**ANS:** The chiasmata, which are visible late in prophase I of meiosis, indicate that chromosomes have crossed over.

**2.15** During meiosis, when does chromosome disjunction occur? When does chromatid disjunction occur?

**ANS:** Chromosome disjunction occurs during anaphase I. Chromatid disjunction occurs during anaphase II.

**2.16** In *Arabidopsis*, is leaf tissue haploid or diploid? How many nuclei are present in the female gametophyte?

How many are present in the male gametophyte? Are these nuclei haploid or diploid?

**ANS:** Leaf tissue is diploid. The female gametophyte contains eight identical haploid nuclei. The male gametophyte contains three identical haploid nuclei.

**2.17** From the information given in Table 2.1 in this chapter, is there a relationship between genome size (measured in base pairs of DNA) and gene number? Explain.

**ANS:** Among eukaryotes, there does not seem to be a clear relationship between genome size and gene number. For example, humans, with 3.2 billion base pairs of genomic DNA, have about 20,500 genes, and *Arabidopsis* plants, with about 150 million base pairs of genomic DNA, have roughly the same number of genes as humans. However, among prokaryotes, gene number is rather tightly correlated with genome size, probably because there is so little nongenic DNA.

**2.18** Are the synergid cells in an *Arabidopsis* female gametophyte genetically identical to the egg cell nestled between them?

**ANS:** Yes.

**2.19** A cell of the bacterium *Escherichia coli*, a prokaryote, contains one chromosome with about 4.6 million base pairs of DNA comprising 4288 protein-encoding genes. A cell of the yeast *Saccharomyces cerevisiae*, a eukaryote, contains about 12 million base pairs of DNA comprising 6268 genes, and this DNA is distributed over 16 distinct chromosomes. Are you surprised that the chromosome of a prokaryote is larger than some of the chromosomes of a eukaryote? Explain your answer.

**ANS:** It is a bit surprising that yeast chromosomes are, on average, smaller than *E. coli* chromosomes because, as a rule, eukaryotic chromosomes are larger than prokaryotic chromosomes. Yeast is an exception because its genome—not quite three times the size of the *E. coli* genome—is distributed over 16 separate chromosomes.

**2.20** Given the way that chromosomes behave during meiosis, is there any advantage for an organism to have an even number of chromosome pairs (such as *Drosophila* does), as opposed to an odd number of chromosome pairs (such as human beings do)?

**ANS:** No, there is no advantage associated with an even number of chromosomes. As long as the chromosomes come in pairs, they will be able to synapse during prophase I and then disjoin during anaphase I to distribute the genetic material properly to the two daughter cells.

**2.21** In flowering plants, two nuclei from the pollen grain participate in the events of fertilization. With which nuclei from the female gametophyte do these nuclei combine? What tissues are formed from the fertilization events?

**ANS:** One of the pollen nuclei fuses with the egg nucleus in the female gametophyte to form the zygote, which then

develops into an embryo and ultimately into a sporophyte. The other genetically functional pollen nucleus fuses with two nuclei in the female gametophyte to form a triploid nucleus, which then develops into a triploid tissue, the endosperm; this tissue nourishes the developing plant embryo.

- 2.22 The mouse haploid genome contains about  $2.9 \times 10^9$  nucleotide pairs of DNA. How many nucleotide pairs of DNA are present in each of the following mouse cells: (a) somatic cell, (b) sperm cell, (c) fertilized egg, (d) primary oocyte, (e) first polar body, and (f) secondary spermatocyte?

**ANS:** (a)  $5.8 \times 10^9$  nucleotide pairs (np); (b)  $2.9 \times 10^9$  np; (c)  $5.8 \times 10^9$  np; (d)  $11.6 \times 10^9$  np; (e)  $5.8 \times 10^9$  np; and (f)  $5.8 \times 10^9$  np

- 2.23 *Arabidopsis* plants have 10 chromosomes (five pairs) in their somatic cells. How many chromosomes are present in each of the following: (a) egg cell nucleus in the female gametophyte, (b) generative cell nucleus in a pollen grain, (c) fertilized endosperm nucleus, and (d) fertilized egg nucleus?

**ANS:** (a) 5, (b) 5, (c) 15, (d) 10.

### CHAPTER 3

- 3.1 On the basis of Mendel's observations, predict the results from the following crosses with peas: (a) a tall (dominant and homozygous) variety crossed with a dwarf variety; (b) the progeny of (a) self-fertilized; (c) the progeny from (a) crossed with the original tall parent; (d) the progeny of (a) crossed with the original dwarf parent.

**ANS:** (a) All tall; (b) 3/4 tall, 1/4 dwarf; (c) all tall; (d) 1/2 tall, 1/2 dwarf.

- 3.2 Mendel crossed pea plants that produced round seeds with those that produced wrinkled seeds and self-fertilized the progeny. In the  $F_2$ , he observed 5474 round seeds and 1850 wrinkled seeds. Using the letters  $W$  and  $w$  for the seed texture alleles, diagram Mendel's crosses, showing the genotypes of the plants in each generation. Are the results consistent with the Principle of Segregation?

**ANS:** Round ( $WW$ )  $\times$  wrinkled ( $ww$ )  $\rightarrow F_1$  round ( $Ww$ );  $F_1$  self-fertilized  $\rightarrow F_2$  3/4 round (2  $WW$ ; 1  $Ww$ ), 1/4 wrinkled ( $ww$ ). The expected results in the  $F_2$  are 5493 round, 1831 wrinkled. To compare the observed and expected results, compute  $\chi^2$  with one degree of freedom;  $(5474 - 5493)^2/5493 = (1850 - 1831)^2/1831 = 0.263$ , which is not significant at the 5% level. Thus, the results are consistent with the Principle of Segregation.

- 3.3 A geneticist crossed wild, gray-colored mice with white (albino) mice. All the progeny were gray. These progeny were intercrossed to produce an  $F_2$ , which consisted of 198 gray and 72 white mice. Propose a hypothesis to

explain these results, diagram the crosses, and compare the results with the predictions of the hypothesis.

**ANS:** The data suggest that coat color is controlled by a single gene with two alleles,  $C$  (gray) and  $c$  (albino), and that  $C$  is dominant over  $c$ . On this hypothesis, the crosses are gray ( $CC$ )  $\times$  albino ( $cc$ )  $\rightarrow F_1$  gray ( $Cc$ );  $F_1 \times F_1 \rightarrow 3/4$  gray (2  $CC$ : 1  $Cc$ ), 1/4 albino ( $cc$ ). The expected results in the  $F_2$  are 203 gray and 67 albino. To compare the observed and expected results, compute  $\chi^2$  with one degree of freedom:  $(198 - 203)^2/203 + (67 - 72)^2/72 = 0.470$ , which is not significant at the 5% level. Thus, the results are consistent with the hypothesis.

- 3.4 A woman has a rare abnormality of the eyelids called ptosis, which prevents her from opening her eyes completely. This condition is caused by a dominant allele,  $P$ . The woman's father had ptosis, but her mother had normal eyelids. Her father's mother had normal eyelids.

(a) What are the genotypes of the woman, her father, and her mother?

(b) What proportion of the woman's children will have ptosis if she marries a man with normal eyelids?

**ANS:** (a) Woman's genotype  $Pp$ , father's genotype  $Pp$ , mother's genotype  $pp$ ; (b)  $1/2$

- 3.5 In pigeons, a dominant allele  $C$  causes a checkered pattern in the feathers; its recessive allele  $c$  produces a plain pattern. Feather coloration is controlled by an independently assorting gene; the dominant allele  $B$  produces red feathers, and the recessive allele  $b$  produces brown feathers. Birds from a true-breeding checkered, red variety are crossed to birds from a true-breeding plain, brown variety.

(a) Predict the phenotype of their progeny.

(b) If these progeny are intercrossed, what phenotypes will appear in the  $F_2$  and in what proportions?

**ANS:** (a) Checkered, red ( $CC BB$ )  $\times$  plain, brown ( $cc bb$ )  $\rightarrow F_1$  all checkered, red ( $Cc Bb$ ); (b)  $F_2$  progeny: 9/16 checkered, red ( $C- B-$ ), 3/16 plain, red ( $cc B-$ ), 3/16 checkered, brown ( $C- bb$ ), 1/16 plain, brown ( $cc bb$ ).

- 3.6 In mice, the allele  $C$  for colored fur is dominant over the allele  $c$  for white fur, and the allele  $V$  for normal behavior is dominant over the allele  $v$  for waltzing behavior, a form of dis-coordination. Given the genotypes of the parents in each of the following crosses:

(a) Colored, normal mice mated with white, normal mice produced 29 colored, normal, and 10 colored, waltzing progeny

(b) Colored, normal mice mated with colored, normal mice produced 38 colored, normal, 15 colored, waltzing, 11 white, normal, and 4 white, waltzing progeny

(c) Colored, normal mice mated with white, waltzing mice produced 8 colored, normal, 7 colored, waltzing, 9 white, normal, and 6 white, waltzing progeny.

**ANS:** (a) colored, normal ( $CC Vv$ )  $\times$  white, normal ( $cc Vv$ )  
 (b) colored, normal ( $Cc Vv$ )  $\times$  colored, normal ( $Cc Vv$ );  
 (c) colored, normal ( $Cc Vv$ )  $\times$  white, waltzing ( $cc vv$ ).

**3.7** In rabbits, the dominant allele  $B$  causes black fur and the recessive allele  $b$  causes brown fur; for an independently assorting gene, the dominant allele  $R$  causes long fur and the recessive allele  $r$  (for *rex*) causes short fur. A homozygous rabbit with long, black fur is crossed with a rabbit with short, brown fur, and the offspring are intercrossed. In the  $F_2$ , what proportion of the rabbits with long, black fur will be homozygous for both genes?

**ANS:** Among the  $F_2$  progeny with long, black fur, the genotypic ratio is 1  $BB RR$ : 2  $BB Rr$ : 2  $Bb RR$ : 4  $Bb Rr$ ; thus, 1/9 of the rabbits with long, black fur are homozygous for both genes.

**3.8** In shorthorn cattle, the genotype  $RR$  causes a red coat, the genotype  $rr$  causes a white coat, and the genotype  $Rr$  causes a roan coat. A breeder has red, white, and roan cows and bulls. What phenotypes might be expected from the following matings and in what proportions?

- (a) Red  $\times$  red
- (b) Red  $\times$  roan
- (c) Red  $\times$  white
- (d) Roan  $\times$  roan.

**ANS:** (a) All red; (b) 1/2 red, 1/2 roan; (c) all roan; (d) 1/4 red, 1/2 roan, 1/4 white

**3.9** How many different kinds of  $F_1$  gametes,  $F_2$  genotypes, and  $F_2$  phenotypes would be expected from the following crosses:

- (a)  $AA \times aa$ ;
- (b)  $AA BB \times aa bb$ ;
- (c)  $AA BB CC \times aa bb cc$ ?
- (d) What general formulas are suggested by these answers?

**ANS:**

$F_1$ Gametes	$F_2$ Genotypes	$F_2$ Phenotypes
(a) 2	3	2
(b) $2 \times 2 = 4$	$3 \times 3 = 9$	$2 \times 2 = 4$
(c) $2 \times 2 \times 2 = 8$	$3 \times 3 \times 3 = 27$	$2 \times 2 \times 2 = 8$
(d) $2^n$	$3^n$	$2^n$ , where $n$ is the number of genes

**3.10** A researcher studied six independently assorting genes in a plant. Each gene has a dominant and a recessive allele:  $R$  black stem,  $r$  red stem;  $D$  tall plant,  $d$  dwarf plant;  $C$  full pods,  $c$  constricted pods;  $O$  round fruit,  $o$  oval fruit;  $H$  hairless leaves,  $h$  hairy leaves;  $W$  purple flower,  $w$  white flower. From the cross (P1)  $Rr Dd cc Oo Hh Ww \times$  (P2)  $Rr dd Cc oo Hh ww$ ,

- (a) How many kinds of gametes can be formed by P1?
- (b) How many genotypes are possible among the progeny of this cross?
- (c) How many phenotypes are possible among the progeny?
- (d) What is the probability of obtaining the  $Rr Dd cc Oo hh ww$  genotype in the progeny?
- (e) What is the probability of obtaining a black, dwarf, constricted, oval, hairy, purple phenotype in the progeny?

**ANS:** (a)  $2 \times 2 \times 1 \times 2 \times 2 \times 2 = 32$ ; (b)  $3 \times 2 \times 2 \times 2 \times 3 \times 2 = 144$ ; (c)  $2 \times 2 \times 2 \times 2 \times 2 \times 2 = 64$ ; (d)  $(1/2) \times (1/2) \times (1/2) \times (1/4) \times (1/2) = 1/128$ ; (e)  $(3/4) \times (1/2) \times (1/2) \times (1/2) \times (1/4) \times (1/2) = 3/256$ .

**3.11** For each of the following situations, determine the degrees of freedom associated with the  $\chi^2$  statistic and decide whether or not the observed  $\chi^2$  value warrants acceptance or rejection of the hypothesized genetic ratio.

Hypothesized Ratio	Observed- $\chi^2$
(a) 3:1	7.0
(b) 1:2:1	7.0
(c) 1:1:1:1	7.0
(d) 9:3:3:1	5.0

**ANS:** (a) 1, reject; (b) 2, reject; (c) 3, accept; (d) 3, accept.

**3.12** Mendel testcrossed pea plants grown from yellow, round  $F_1$  seeds to plants grown from green, wrinkled seeds and obtained the following results: 31 yellow, round; 26 green, round; 27 yellow, wrinkled; and 26 green, wrinkled. Are these results consistent with the hypothesis that seed color and seed texture are controlled by independently assorting genes, each segregating two alleles?

**ANS:** On the hypothesis, the expected number in each class is 27.5;  $\chi^2$  with three degrees of freedom is calculated as  $(31 - 27.5)^2/27.5 + (26 - 27.5)^2/27.5 + (27 - 27.5)^2/27.5 + (26 - 27.5)^2/27.5 = 0.618$ , which is not significant at the 5% level. Thus, the results are consistent with the hypothesis of two independently assorting genes, each segregating two alleles.

**3.13** Perform a chi-square test to determine if an observed ratio of 30 tall to 20 dwarf pea plants is consistent with an expected ratio of 1:1 from the cross  $Dd \times dd$ .

**ANS:**  $\chi^2 = (30 - 25)^2/25 + (20 - 25)^2/25 = 2$ , which is less than 3.84, the 5 percent critical value for a chi-square statistic with one degree of freedom; consequently, the observed segregation ratio is consistent with the expected ratio of 1:1.

**3.14** Seed capsules of the Shepherd's purse are either triangular or ovoid. A cross between a plant with triangular seed capsules and a plant with ovoid seed capsules yielded  $F_1$  hybrids that all had triangular seed capsules. When these  $F_1$  hybrids were intercrossed, they produced 80  $F_2$  plants, 72 of which had triangular seed capsules and 8 of which had ovoid seed capsules. Are these results consistent with the hypothesis that capsule shape is determined by a single gene with two alleles?

**ANS:** If capsule shape is determined by a single gene with two alleles, the  $F_2$  plants should segregate in a 3:1 ratio. To test for agreement between the observed segregation data and the expected ratio, compute the expected number of plants with either triangular or ovoid seed capsules:  $(3/4) \times 80 = 60$  triangular and  $(1/4) \times 80 = 20$  ovoid; then compute a  $\chi^2$  statistic with one degree of freedom:  $\chi^2 = (72 - 60)^2/60 + (8 - 20)^2/20 = 9.6$ , which exceeds the critical value of 3.84. Consequently, the data are inconsistent with the hypothesis that capsule shape is determined by a single gene with two alleles.

**3.15** Albinism in humans is caused by a recessive allele  $a$ . From marriages between people known to be carriers ( $Aa$ ) and people with albinism ( $aa$ ), what proportion of the children would be expected to have albinism? Among three children, what is the chance of one without albinism and two with albinism?

**ANS:** Half the children from  $Aa \times aa$  matings would have albinism. In a family of three children, the chance that one will be unaffected and two affected is  $3 \times (1/2)^1 \times (1/2)^2 = 3/8$ .

**3.16** If both husband and wife are known to be carriers of the allele for albinism, what is the chance of the following combinations in a family of four children: (a) all four unaffected; (b) three unaffected and one affected; (c) two unaffected and two affected; (d) one unaffected and three affected?

**ANS:** (a)  $(3/4)^4 = 81/256$ ; (b)  $4 \times (3/4)^3 \times (1/4)^1 = 108/256$ ; (c)  $6 \times (3/4)^2 \times (1/4)^2 = 54/256$ ; (d)  $4 \times (3/4)^1 \times (1/4)^3 = 12/256$ .

**3.17** In humans, cataracts in the eyes and fragility of the bones are caused by dominant alleles that assort independently. A man with cataracts and normal bones marries a woman without cataracts but with fragile bones. The man's father had normal eyes, and the woman's father had normal bones. What is the probability that the first child of this couple will (a) be free from both abnormalities; (b) have cataracts but not have fragile bones; (c) have fragile bones but not have cataracts; (d) have both cataracts and fragile bones?

**ANS:** Man ( $Cc ff$ )  $\times$  woman ( $cc Ff$ ). (a)  $cc ff$ ,  $(1/2) \times (1/2) = 1/4$ ; (b)  $Cc ff$ ,  $(1/2) \times (1/2) = 1/4$ ; (c)  $cc Ff$ ,  $(1/2) \times (1/2) = 1/4$ ; (d)  $Cc Ff$ ,  $(1/2) \times (1/2) = 1/4$ .

**3.18** In generation V in the pedigree in Figure 3.15, what is the probability of observing seven children without the cancer-causing mutation and two children with this mutation among a total of nine children?

**ANS:**  $9!/(7! 2!) \times (1/2)^7 \times (1/2)^2 = 0.07$

**3.19** If a man and a woman are heterozygous for a gene, and if they have three children, what is the chance that all three will also be heterozygous?

**ANS:**  $(1/2)^3 = 1/8$

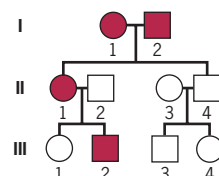
**3.20** If four babies are born on a given day: (a) What is the chance that two will be boys and two will be girls? (b) What is the chance that all four will be girls? (c) What combination of boys and girls among four babies is most likely? (d) What is the chance that at least one baby will be a girl?

**ANS:** (a)  $4 \times (1/2)^2 \times (1/2)^2 = 4/16$ ; (b)  $(1/2)^4 = 1/16$ ; (c) 2 boys, girls; (d)  $1 - \text{probability that all four are boys} = 1 - (1/2)^4 = 15/16$ .

**3.21** In a family of six children, what is the chance that at least three are girls?

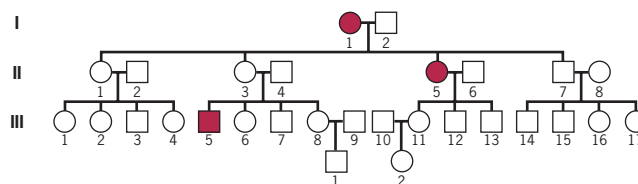
**ANS:**  $(20/64) + (15/64) + (6/64) + (1/64) = 42/64$

**3.22** The following pedigree shows the inheritance of a dominant trait. What is the chance that the offspring of the following matings will show the trait: (a) III-1  $\times$  III-3; (b) III-2  $\times$  III-4?



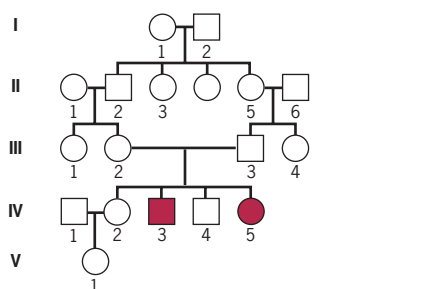
**ANS:** (a) zero; (b) 1/2

**3.23** The following pedigree shows the inheritance of a recessive trait. Unless there is evidence to the contrary, assume that the individuals who have married into the family do not carry the recessive allele. What is the chance that the offspring of the following matings will show the trait: (a) III-1  $\times$  III-12; (b) II-4  $\times$  III-14; (c) III-6  $\times$  III-13; (d) IV-1  $\times$  IV-2?

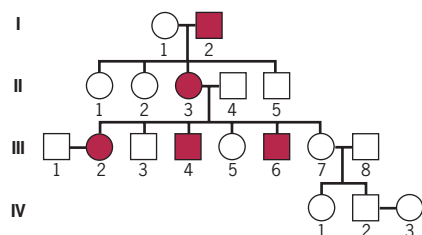


**ANS:** (a)  $(1/2) \times (1/4) = 1/8$ ; (b)  $(1/2) \times (1/2) \times (1/4) = 1/16$ ; (c)  $(2/3) \times (1/4) = 1/6$ ; (d)  $(2/3) \times (1/2) \times (1/2) \times (1/4) = 1/24$

- 3.24 In the following pedigrees, determine whether the trait is more likely to be due to a dominant or a recessive allele. Assume the trait is rare in the population.



(a)



(b)

**ANS:** (a) Recessive; (b) dominant.

- 3.25 In pedigree (b) of Problem 3.24, what is the chance that the couple III-1 and III-2 will have an affected child? What is the chance that the couple IV-2 and IV-3 will have an affected child?

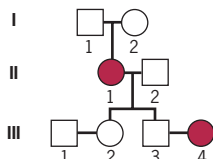
**ANS:** For III-1  $\times$  III-2, the chance of an affected child is  $1/2$ . For IV-2  $\times$  IV-3, the chance is zero.

- 3.26 Peas heterozygous for three independently assorting genes were intercrossed.

- What proportion of the offspring will be homozygous for all three recessive alleles?
- What proportion of the offspring will be homozygous for all three genes?
- What proportion of the offspring will be homozygous for one gene and heterozygous for the other two?
- What proportion of the offspring will be homozygous for the recessive allele of at least one gene?

**ANS:** (a)  $(1/4)^3 = 1/64$ ; (b)  $(1/2)^3 = 1/8$ ; (c)  $3 \times (1/2)^1 \times (1/2)^2 = 3/8$ ; (d)  $1 - \text{probability that the offspring is not homozygous for the recessive allele of any gene} = 1 - (3/4)^3 = 37/64$ .

- 3.27 The following pedigree shows the inheritance of a recessive trait. What is the chance that the couple III-3 and III-4 will have an affected child?



**ANS:**  $1/2$

- 3.28 A geneticist crosses tall pea plants with short pea plants. All the  $F_1$  plants are tall. The  $F_1$  plants are then allowed to self-fertilize, and the  $F_2$  plants are classified by height: 62 tall and 26 short. From these results, the geneticist concludes that shortness in peas is due to a recessive allele ( $s$ ) and that tallness is due to a dominant allele ( $S$ ). On this hypothesis,  $2/3$  of the tall  $F_2$  plants should be heterozygous  $Ss$ . To test this prediction, the geneticist uses pollen from each of the 62 tall plants to fertilize the ovules of emasculated flowers on short pea plants. The next year, three seeds from each of the 62 crosses are sown in the garden and the resulting plants are grown to maturity. If none of the three plants from a cross is short, the male parent is classified as having been homozygous  $SS$ ; if at least one of the three plants from a cross is short, the male parent is classified as having been heterozygous  $Ss$ . Using this system of progeny testing, the geneticist concludes that 29 of the 62 tall  $F_2$  plants were homozygous  $SS$  and that 33 of these plants were heterozygous  $Ss$ .

(a) Using the chi-square procedure, evaluate these results for goodness of fit to the prediction that  $2/3$  of the tall  $F_2$  plants should be heterozygous.

(b) Informed by what you read in *A Milestone in Genetics: Mendel's 1866 Paper*, which you can find in the Student Companion Site, explain why the geneticist's procedure for classifying tall  $F_2$  plants by genotype is not definitive.

(c) Adjust for the uncertainty in the geneticist's classification procedure and calculate the expected frequencies of homozygotes and heterozygotes among the tall  $F_2$  plants.

(d) Evaluate the predictions obtained in (c) using the chi-square procedure.

**ANS:** (a) The observed numbers, expected numbers, and chi-square calculation are laid out in the following table:

	Observed	Expected	$(\text{Obs} - \text{Exp})^2 / \text{Exp}$
Dominant homozygotes ( $SS$ )	29	$62 \times 1/3 = 20.7$	3.33
Heterozygotes ( $Ss$ )	33	$62 \times 2/3 = 41.3$	1.67
Total	62	62	5.00

The total chi-square value is greater than the critical value for a chi-square statistic with one degree of freedom (3.84). Therefore, we reject the hypothesis that the expected proportions are  $1/3$  and  $2/3$ .

(b) The problem with the geneticist's classification procedure is that it allows for a heterozygote to be

misclassified as a homozygote if none of its three progeny shows the recessive (short) phenotype. The probability of this event is  $1/2$  for any one offspring—therefore  $(1/2)^3 = 1/8$  for all three offspring.

(c) The predicted frequencies must take into account the probability of misclassifying a heterozygote as a homozygote. The frequency of heterozygotes expected *a priori* ( $2/3$ ) must be decreased by the probability of misclassification ( $1/8$ ); thus, the predicted frequency of heterozygotes is  $62 \times (2/3) \times (1 - 1/8) = 62 \times (7/12) = 36.2$ . The predicted frequency of homozygotes is obtained by subtraction:  $62 - 36.2 = 25.8$ .

(d) The chi-square calculation is  $(29 - 25.8)^2/25.8 + (33 - 36.2)^2/36.2 = 0.68$ , which is much less than the critical value for a chi-square statistic with one degree of freedom. Therefore, we tentatively accept the idea that adjusting for the probability of misclassification explains the observed data.

- 3.29 A researcher who has been studying albinism has identified a large group of families with four children in which at least one child shows albinism. None of the parents in this group of families shows albinism. Among the children, the ratio of those without albinism to those with albinism is 1.7:1. The researcher is surprised by this result because he thought that a 3:1 ratio would be expected on the basis of Mendel's Principle of Segregation. Can you explain the apparently non-Mendelian segregation ratio in the researcher's data?

**ANS:** The researcher has obtained what appears to be a non-Mendelian ratio because he has been studying only families in which at least one child shows albinism. In these families, both parents are heterozygous for the mutant allele that causes albinism. However, other couples in the population might also be heterozygous for this allele but, simply due to chance, have failed to produce a child with albinism. If a man and a woman are both heterozygous carriers of the mutant allele, the chance that a child they produce will not have albinism is  $3/4$ . The chance that four children they produce will not have albinism is therefore  $(3/4)^4 = 0.316$ . In the entire population of families in which two heterozygous parents have produced a total of four children, the average number of affected children is 1. Among families in which two heterozygous parents have produced at least one affected child among a total of four children, the average must be greater than 1. To calculate this *conditional average*, let us denote the number of children with albinism by  $x$ , and the probability that exactly  $x$  of the four children have albinism by  $P(x)$ . The average number of affected children among families in which at least one of the four children is affected—that is, the conditional average—is therefore  $\sum xP(x)/(1 - P(0))$ , where the sum starts at  $x = 1$  and ends at  $x = 4$ . We start the sum at  $x = 1$  because we must exclude those cases in which none of the four children is affected. The divisor  $(1 - P(0))$  is the probability that the couple has had at least one affected child among their

four children. Now  $P(0) = 0.316$  and  $\sum xP(x) = 1$ . Therefore, the average we seek is simply  $1/(1 - 0.316) = 1.46$ . If, in the subset of families with at least one affected child, the average number of affected children is 1.46, then the average number of unaffected children is  $4 - 1.46 = 2.54$ . Thus, the expected ratio of unaffected to affected children in these families is 2.54:1.46, or 1.74:1, which is what the researcher has observed.

## CHAPTER 4

- 4.1 What blood types could be observed in children born to a woman who has blood type M and a man who has blood type MN?

**ANS:** M and MN.

- 4.2 In rabbits, coloration of the fur depends on alleles of the gene  $c$ . From information given in the chapter, what phenotypes and proportions would be expected from the following crosses: (a)  $c^+c^+ \times cc$ ; (b)  $c^+c \times c^+c$ ; (c)  $c^+c^b \times c^+c^b$ ; (d)  $cc^b \times cc$ ; (e)  $c^+c^b \times c^+c$ ; (f)  $c^bc \times cc$ ?

**ANS:** (a) All wild-type; (b)  $3/4$  wild-type,  $1/4$  albino; (c)  $3/4$  wild-type,  $1/4$  chinchilla; (d)  $1/2$  chinchilla,  $1/2$  albino; (e)  $3/4$  wild-type,  $1/4$  Himalayan; (f)  $1/2$  Himalayan,  $1/2$  albino.

- 4.3 In mice, a series of five alleles determines fur color. In order of dominance, these alleles are as follows:  $A^Y$ , yellow fur but homozygous lethal;  $A^L$ , agouti with light belly;  $A^+$ , agouti (wild-type);  $a^t$ , black and tan; and  $a$ , black. For each of the following crosses, give the coat color of the parents and the phenotypic ratios expected among the progeny: (a)  $A^YA^L \times A^YA^L$ ; (b)  $A^YA \times A^L a^t$ ; (c)  $a^t a \times A^Y a$ ; (d)  $A^L a^t \times A^L A^L$ ; (e)  $A^L A^L \times A^Y A^+$ ; (f)  $A^+ a^t \times a^t a$ ; (g)  $a^t a \times aa$ ; (h)  $A^Y A^L \times A^+ a^t$ ; and (i)  $A^Y a^L \times A^Y A^+$ .

**ANS:**

	Parents	Offspring
(a)	Yellow $\times$ yellow	2 yellow: 1 light belly
(b)	Yellow $\times$ light belly	2 yellow: 1 light belly: 1 black and tan
(c)	Black and tan $\times$ yellow	2 yellow: 1 black and tan: 1 black
(d)	Light belly $\times$ light belly	All light belly
(e)	Light belly $\times$ yellow	1 yellow: 1 light belly
(f)	Agouti $\times$ black and tan	1 agouti: 1 black and tan
(g)	Black and tan $\times$ black	1 black and tan: 1 black
(h)	Yellow $\times$ agouti	1 yellow: 1 light belly
(i)	Yellow $\times$ yellow	2 yellow: 1 light belly

- 4.4 In several plants, such as tobacco, primrose, and red clover, combinations of alleles in eggs and pollen have been found to influence the reproductive compatibility of the plants. Homozygous combinations, such as  $S^1 S^1$ , do not



develop because  $S^1$  pollen is not effective on  $S^1$  stigmas. However,  $S^1$  pollen is effective on  $S^2S^3$  stigmas. What progeny might be expected from the following crosses (seed parent written first): (a)  $S^1S^2 \times S^2S^3$ ; (b)  $S^1S^2 \times S^3S^4$ ; (c)  $S^4S^5 \times S^4S^5$ ; and (d)  $S^3S^4 \times S^5S^6$ ?

**ANS:** (a)  $S^1S^2, S^1S^3, S^2S^3$ ; (b)  $S^1S^3, S^1S^4, S^2S^3, S^2S^4$ ; (c)  $S^4S^5$ ; (d)  $S^3S^5, S^3S^6, S^4S^5, S^4S^6$ .

**4.5** From information in the chapter about the ABO blood types, what phenotypes and ratios are expected from the following matings: (a)  $I^A I^A \times I^B I^B$ ; (b)  $I^A I^B \times ii$ ; (c)  $I^A i \times I^B i$ ; and (d)  $I^A i \times ii$ ;

**ANS:** (a) All AB; (b) 1 A: 1 B; (c) 1 A: 1 B: 1 AB: 1 O; (d) 1 A: 1 O.

**4.6** A woman with type O blood gave birth to a baby, also with type O blood. The woman stated that a man with type AB blood was the father of the baby. Is there any merit to her statement?

**ANS:** No. The woman must be  $ii$ ; if her mate is  $I^A I^B$ ; they could not have an  $ii$  child.

**4.7** A woman with type AB blood gave birth to a baby with type B blood. Two different men claim to be the father. One has type A blood, the other has type B blood. Can the genetic evidence decide in favor of either?

**ANS:** No. The woman is  $I^A I^B$ . One man could be either  $I^A I^A$  or  $I^A i$ ; the other could be either  $I^B I^B$  or  $I^B i$ . Given the uncertainty in the genotype of each man, either could be the father of the child.

**4.8** The flower colors of plants in a particular population may be blue, purple, turquoise, light blue, or white. A series of crosses between different members of the population produced the following results:

Cross	Parents	Progeny
1	Purple $\times$ blue	All purple
2	Purple $\times$ purple	76 purple, 25 turquoise
3	Blue $\times$ blue	86 blue, 29 turquoise
4	Purple $\times$ turquoise	49 purple, 52 turquoise
5	Purple $\times$ purple	69 purple, 22 blue
6	Purple $\times$ blue	50 purple, 51 blue
7	Purple $\times$ blue	54 purple, 26 blue, 25 turquoise
8	Turquoise $\times$ turquoise	All turquoise
9	Purple $\times$ blue	49 purple, 25 blue, 23 light blue
10	Light blue $\times$ light blue	60 light blue, 29 turquoise, 31 white
11	Turquoise $\times$ white	All light blue
12	White $\times$ white	All white
13	Purple $\times$ white	All purple

How many genes and alleles are involved in the inheritance of flower color? Indicate all possible genotypes for the following phenotypes: (a) purple; (b) blue; (c) turquoise; (d) light blue; (e) white.

**ANS:** One gene with four alleles. (a) purple:  $c^p c^p, c^p c^b, c^p c^t, c^p c^w$ ; (b) blue:  $c^b c^b, c^b c^t, c^b c^w$ ; (c) turquoise:  $c^t c^t, c^t c^w$ ; (d) light blue:  $c^t c^w$ ; (e) white:  $c^w c^w$ .

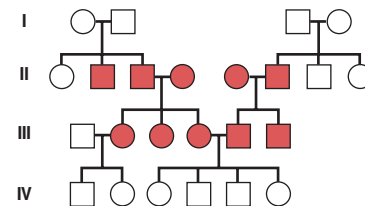
**4.9** A woman who has blood type O and blood type M marries a man who has blood type AB and blood type MN. If we assume that the genes for the A-B-O and M-N blood-typing systems assort independently, what blood types might the children of this couple have, and in what proportions?

**ANS:** The woman is  $ii L^M L^M$ ; the man is  $I^A I^B L^M L^N$ ; the blood types of the children will be A and M, A and MN, B and M, and B and MN, all equally likely.

**4.10** A Japanese strain of mice has a peculiar, uncoordinated gait called waltzing, which is due to a recessive allele,  $v$ . The dominant allele  $V$  causes mice to move in a coordinated manner. A mouse geneticist has recently isolated another recessive mutation that causes uncoordinated movement. This mutation, called *tango*, could be an allele of the *waltzing* gene, or it could be a mutation in an entirely different gene. Propose a test to determine whether the *waltzing* and *tango* mutations are alleles, and if they are, propose symbols to denote them.

**ANS:** Cross homozygous *waltzing* with homozygous *tango*. If the mutations are alleles, all the offspring will have an uncoordinated gait; if they are not alleles, all the offspring will be wild-type. If the two mutations are alleles, they could be denoted with the symbols  $v$  (*waltzing*) and  $v'$  (*tango*).

**4.11** Congenital deafness in human beings is inherited as a recessive condition. In the following pedigree, two deaf individuals, each presumably homozygous for a recessive mutation, have married and produced four children with normal hearing. Propose an explanation.



**ANS:** The individuals III-4 and III-5 must be homozygous for recessive mutations in different genes; that is, one is  $aa BB$  and the other is  $AA bb$ ; none of their children is deaf because all of them are heterozygous for both genes ( $Aa Bb$ ).

**4.12** In the fruit fly, recessive mutations in either of two independently assorting genes, *brown* and *purple*, prevent the synthesis of red pigment in the eyes. Thus, homozygotes for either of these mutations have brownish-purple eyes.

10-WC Answers to All Questions and Problems

However, heterozygotes for both of these mutations have dark red, that is, wild-type eyes. If such double heterozygotes are intercrossed, what kinds of progeny will be produced, and in what proportions?

**ANS:** 9/16 dark red, 7/16 brownish purple.

**4.13** The dominant mutation *Plum* in the fruit fly also causes brownish-purple eyes. Is it possible to determine by genetic experiments whether *Plum* is an allele of the *brown* or *purple* genes?

**ANS:** No. The test for allelism cannot be performed with dominant mutations.

**4.14** From information given in the chapter, explain why mice with yellow coat color are not true-breeding.

**ANS:** The allele for yellow fur is homozygous lethal.

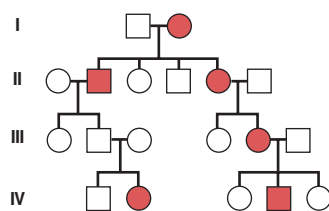
**4.15** A couple has four children. Neither the father nor the mother is bald; one of the two sons is bald, but neither of the daughters is bald.

(a) If one of the daughters marries a nonbald man and they have a son, what is the chance that the son will become bald as an adult?

(b) If the couple has a daughter, what is the chance that she will become bald as an adult?

**ANS:** The mother is *Bb* and the father is *bb*. The chance that a daughter is *Bb* is 1/2. (a) The chance that the daughter will have a bald son is  $(1/2) \times (1/2) = 1/4$ . (b) The chance that the daughter will have a bald daughter is zero.

**4.16** The following pedigree shows the inheritance of ataxia, a rare neurological disorder characterized by uncoordinated movements. Is ataxia caused by a dominant or a recessive allele? Explain.



**ANS:** Dominant. The condition appears in every generation and nearly every affected individual has an affected parent. The exception, IV-2, had a father who carried the ataxia allele but did not manifest the trait—an example of incomplete penetrance.

**4.17** Chickens that carry both the alleles for rose comb (*R*) and pea comb (*P*) have walnut combs, whereas chickens that lack both of these alleles (i.e., they are genotypically *rr pp*) have single combs. From the information about interactions between these two genes given in the chapter, determine the phenotypes and proportions expected from the following crosses:

(a)  $RR Pp \times rr Pp$ ;

(b)  $rr PP \times Rr Pp$ ;

(c)  $Rr Pp \times Rr pp$ ;

(d)  $Rr pp \times rr pp$ .

**ANS:** (a) 3/4 walnut, 1/4 rose; (b) 1/2 walnut, 1/2 pea; (c) 3/8 walnut, 3/8 rose, 1/8 pea, 1/8 single; (d) 1/2 rose, 1/2 single.

**4.18** Rose-comb chickens mated with walnut-comb chickens produced 15 walnut-, 14 rose-, 5 pea-, and 6 single-comb chicks. Determine the genotypes of the parents.

**ANS:**  $Rr pp \times Rr Pp$ .

**4.19** Summer squash plants with the dominant allele *C* bear white fruit, whereas plants homozygous for the recessive allele *c* bear colored fruit. When the fruit is colored, the dominant allele *G* causes it to be yellow; in the absence of this allele (i.e., with genotype *gg*), the fruit color is green. What are the  $F_2$  phenotypes and proportions expected from intercrossing the progeny of *CC GG* and *cc gg* plants? Assume that the *C* and *G* genes assort independently.

**ANS:** 12/16 white, 3/16 yellow, 1/16 green.

**4.20** The white Leghorn breed of chickens is homozygous for the dominant allele *C*, which produces colored feathers. However, this breed is also homozygous for the dominant allele *I* of an independently assorting gene that inhibits coloration of the feathers. Consequently, Leghorn chickens have white feathers. The white Wyandotte breed of chickens has neither the allele for color nor the inhibitor of color; it is therefore genotypically *cc ii*. What are the  $F_2$  phenotypes and proportions expected from intercrossing the progeny of a white Leghorn hen and a white Wyandotte rooster?

**ANS:** 13/16 white, 3/16 colored.

**4.21** Fruit flies homozygous for the recessive mutation *scarlet* have bright red eyes because they cannot synthesize brown pigment. Fruit flies homozygous for the recessive mutation *brown* have brownish-purple eyes because they cannot synthesize red pigment. Fruit flies homozygous for both of these mutations have white eyes because they cannot synthesize either type of pigment. The *brown* and *scarlet* mutations assort independently. If fruit flies that are heterozygous for both of these mutations are intercrossed, what kinds of progeny will they produce, and in what proportions?

**ANS:** 9/16 dark red (wild-type), 3/16 brownish purple, 3/16 bright red, 1/16 white.

**4.22** Consider the following hypothetical scheme of determination of coat color in a mammal. Gene *A* controls the conversion of a white pigment  $P_0$  into a gray pigment

$P_1$ ; the dominant allele  $A$  produces the enzyme necessary for this conversion, and the recessive allele  $a$  produces an enzyme without biochemical activity. Gene  $B$  controls the conversion of the gray pigment  $P_1$  into a black pigment  $P_2$ ; the dominant allele  $B$  produces the active enzyme for this conversion, and the recessive allele  $b$  produces an enzyme without activity. The dominant allele  $C$  of a third gene produces a polypeptide that completely inhibits the activity of the enzyme produced by gene  $A$ ; that is, it prevents the reaction  $P_0 \rightarrow P_1$ . Allele  $c$  of this gene produces a defective polypeptide that does not inhibit the reaction  $P_0 \rightarrow P_1$ . Genes  $A$ ,  $B$ , and  $C$  assort independently, and no other genes are involved. In the  $F_2$  of the cross  $AA\ bb\ CC \times aa\ BB\ cc$ , what is the expected phenotypic segregation ratio?

**ANS:** 9 black: 3 gray: 52 white.

- 4.23** What  $F_2$  phenotypic segregation ratio would be expected for the cross described in the preceding problem if the dominant allele,  $C$ , of the third gene produced a product that completely inhibited the activity of the enzyme produced by gene  $B$ —that is, prevented the reaction  $P_1 \rightarrow P_2$ , rather than inhibiting the activity of the enzyme produced by gene  $A$ ?

**ANS:** 9 black: 39 gray: 16 white.

- 4.24** The Micronesian Kingfisher, *Halcyon cinnamomina*, has a cinnamon-colored face. In some birds, the color continues onto the chest, producing one of three patterns: a circle, a shield, or a triangle; in other birds, there is no color on the chest. A male with a colored triangle was crossed with a female that had no color on her chest, and all their offspring had a colored shield on the chest. When these offspring were intercrossed, they produced an  $F_2$  with a phenotypic ratio of 3 circle: 6 shield: 3 triangle: 4 no color. (a) Determine the mode of inheritance for this trait and indicate the genotypes of the birds in all three generations. (b) If a male without color on his chest is mated to a female with a colored shield on her chest and the  $F_1$  segregate in the ratio of 1 circle: 2 shield: 1 triangle, what are the genotypes of the parents and their progeny?

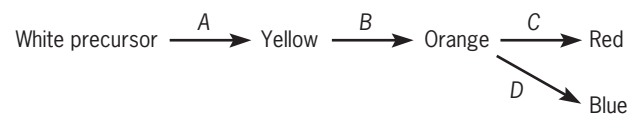
(a) The simplest explanation for the inheritance of the trait is recessive epistasis combined with incomplete dominance, summarized in the following table:

Genotype	Phenotype	Frequency in $F_2$
$AA\ B-$	Circle	3/16
$Aa\ B-$	Shield	6/16
$aa\ B-$	Triangular	3/16
$A-\ bb$	No color	3/16
$aa\ bb$	No color	1/16

- (b) Father's genotype:  $Aa\ bb$ ; mother's genotype:  $Aa\ BB$

	Circle	Shield	Triangle
Progeny genotypes:	$AA\ Bb$	$Aa\ Bb$	$aa\ Bb$

- 4.25** In a species of tree, seed color is determined by four independently assorting genes:  $A$ ,  $B$ ,  $C$ , and  $D$ . The recessive alleles of each of these genes ( $a$ ,  $b$ ,  $c$ , and  $d$ ) produce abnormal enzymes that cannot catalyze a reaction in the biosynthetic pathway for seed pigment. This pathway is diagrammed as follows:



When both red and blue pigments are present, the seeds are purple. Trees with the genotypes  $Aa\ Bb\ Cc\ Dd$  and  $Aa\ Bb\ Cc\ dd$  were crossed.

- (a) What color are the seeds in these two parental genotypes?  
 (b) What proportion of the offspring from the cross will have white seeds?  
 (c) Determine the relative proportions of red, white, and blue offspring from the cross.

**ANS:** (a) Purple  $\times$  red; (b) proportion white ( $aa$ ) = 1/4; (c) proportion red ( $A-\ B-\ C-\ dd$ ) =  $(3/4)(3/4)(3/4)(1/2) = 27/128$ , proportion white ( $aa$ ) =  $1/4 = 32/128$ , proportion blue ( $A-\ B-\ cc\ Dd$ ) =  $(3/4)(3/4)(1/4)(1/2) = 9/128$ .

- 4.26** Multiple crosses were made between true-breeding lines of black and yellow Labrador retrievers. All the  $F_1$  progeny were black. When these progeny were intercrossed, they produced an  $F_2$  consisting of 91 black, 39 yellow, and 30 chocolate. (a) Propose an explanation for the inheritance of coat color in Labrador retrievers. (b) Propose a biochemical pathway for coat color determination and indicate how the relevant genes control coat coloration.

**ANS:** (a) Because the  $F_2$  segregation is approximately 9 black: 3 chocolate: 4 yellow, coat color is determined by epistasis between two independently assorting genes: black =  $B-\ E-$ ; chocolate =  $bb\ E-$ ; yellow =  $B-\ ee$  or  $bb\ ee$ . (b) Yellow pigment— $E \rightarrow$  brown pigment— $B \rightarrow$  black pigment.

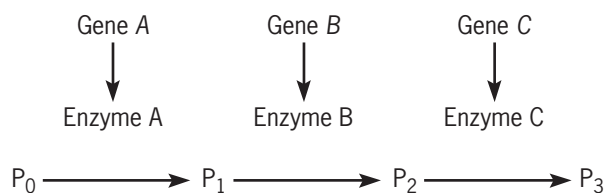
- 4.27** Two plants with white flowers, each from true-breeding strains, were crossed. All the  $F_1$  plants had red flowers. When these  $F_1$  plants were intercrossed, they produced an  $F_2$  consisting of 177 plants with red flowers and

12-WC Answers to All Questions and Problems

142 with white flowers. (a) Propose an explanation for the inheritance of flower color in this plant species. (b) Propose a biochemical pathway for flower pigmentation and indicate which genes control which steps in this pathway.

**ANS:** (a) Because the  $F_2$  segregation is approximately 9 red: 7 white, flower color is due to epistasis between two independently assorting genes: red =  $A- B-$  and white =  $aa B-$ ,  $A- bb$ , or  $aa bb$ . (b) Colorless precursor  $\xrightarrow{A}$  colorless product  $\xrightarrow{B}$  red pigment.

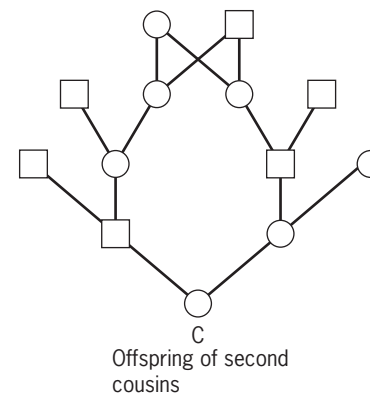
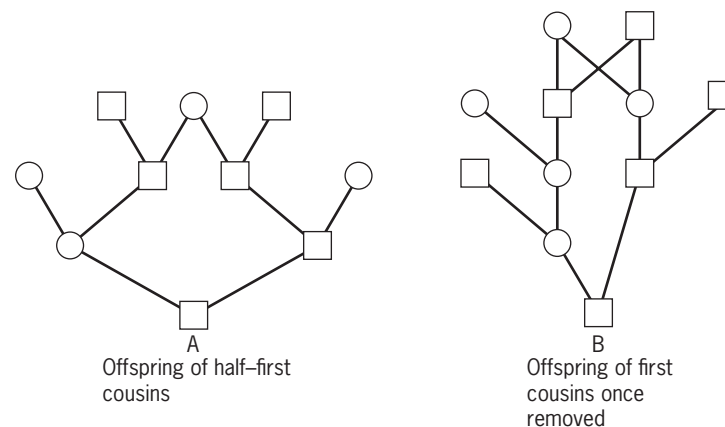
**4.28** Consider the following genetically controlled biosynthetic pathway for pigments in the flowers of a hypothetical plant:



Assume that gene  $A$  controls the conversion of a white pigment,  $P_0$ , into another white pigment,  $P_1$ ; the dominant allele  $A$  specifies an enzyme necessary for this conversion, and the recessive allele  $a$  specifies a defective enzyme without biochemical function. Gene  $B$  controls the conversion of the white pigment,  $P_1$ , into a pink pigment,  $P_2$ ; the dominant allele,  $B$ , produces the enzyme necessary for this conversion, and the recessive allele,  $b$ , produces a defective enzyme. The dominant allele,  $C$ , of the third gene specifies an enzyme that converts the pink pigment,  $P_2$ , into a red pigment,  $P_3$ ; its recessive allele,  $c$ , produces an altered enzyme that cannot carry out this conversion. The dominant allele,  $D$ , of a fourth gene produces a polypeptide that completely inhibits the function of enzyme  $C$ ; that is, it blocks the reaction  $P_2 \rightarrow P_3$ . Its recessive allele,  $d$ , produces a defective polypeptide that does not block this reaction. Assume that flower color is determined solely by these four genes and that they assort independently. In the  $F_2$  of a cross between plants of the genotype  $AA bb CC DD$  and plants of the genotype  $aa BB cc dd$ , what proportion of the plants will have (a) red flowers? (b) pink flowers? (c) white flowers?

**ANS:** (a) Proportion red =  $(3/4)^3 \times (1/4) = 27/256$ ; (b) proportion pink =  $(3/4)^4 + [(3/4)^2 \times (1/4)] = 117/256$ ; (c) proportion white =  $1 - 144/256 = 112/256$ .

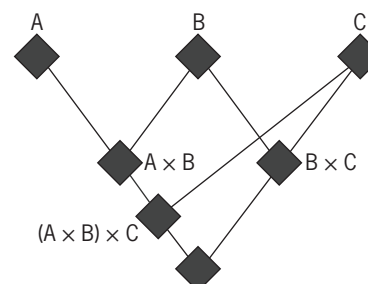
**4.29** In the following pedigrees, what are the inbreeding coefficients of A, B, and C?



**ANS:**  $F_A = (1/2)^5 = 1/32$ ;  $F_B = 2 \times (1/2)^6 = 1/32$ ;  $F_C = 2 \times (1/2)^7 = 1/64$ .

**4.30** A, B, and C are inbred strains of mice, assumed to be completely homozygous. A is mated to B and B to C. Then the  $A \times B$  hybrids are mated to C, and the offspring of this mating are mated to the  $B \times C$  hybrids. What is the inbreeding coefficient of the offspring of this last mating?

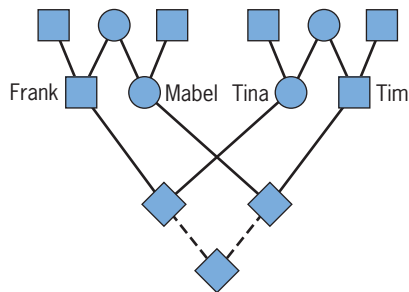
**ANS:** From the following pedigree, the inbreeding coefficient is  $(1/2)^3 (1 + F_C) + (1/2)^4 (1 + F_B) = 3/8$  because  $F_B = F_C = 1$ .



**4.31** Mabel and Frank are half siblings, as are Tina and Tim. However, these two pairs of half siblings do not have any common ancestors. If Mabel marries Tim and Frank marries Tina and each couple has a child, what fraction of their genes will these children share by virtue of

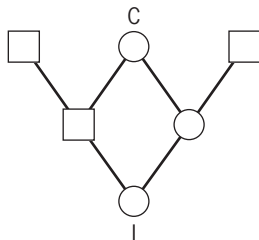
common ancestry? Will the children be more or less closely related than first cousins?

**ANS:** The pedigree is shown below:



The coefficient of relationship between the offspring of the two couples is obtained by calculating the inbreeding coefficient of the imaginary child from a mating between these offspring and multiplying by 2:  $[(1/2)^5 \times 2] \times 2 = 1/8$ . This is the same degree of relatedness as first cousins.

- 4.32** Suppose that the inbreeding coefficient of I in the following pedigree is 0.25. What is the inbreeding coefficient of I's common ancestor, C?



**ANS:**  $F_I = (1/2)^3(1 + F_C) = 0.25$ ; thus,  $F_C = 1$ .

- 4.33** A randomly pollinated strain of maize produces ears that are 24 cm long, on average. After one generation of self-fertilization, the ear length is reduced to 20 cm. Predict the ear length if self-fertilization is continued for one more generation.

**ANS:** The mean ear length for randomly mated maize is 24 cm and that for maize from one generation of self-fertilization is 20 cm. The inbreeding coefficient of the offspring of one generation of self-fertilization is  $1/2$ , and the inbreeding coefficient of the offspring of two generations of self-fertilization is  $(1/2)(1+1/2) = 3/4$ . Mean ear length ( $Y$ ) is expected to decline linearly with inbreeding according to the equation  $Y = 24 - b F_I$  where  $b$  is the slope of the line. The value of  $b$  can be determined from the two values of  $Y$  that are given. The difference between these two values (4 cm) corresponds to an increase in  $F$  from 0 to  $1/2$ . Thus,  $b = 4/(1/2) = 8$  cm, and for  $F = 3/4$ , the predicted mean ear length is  $Y = 24 - 8 \times (3/4) = 18$  cm.

## CHAPTER 5

- 5.1** What are the genetic differences between male- and female-determining sperm in animals with heterogametic males?

**ANS:** The male-determining sperm carries a Y chromosome; the female-determining sperm carries an X chromosome.

- 5.2** A male with singed bristles appeared in a culture of *Drosophila*. How would you determine if this unusual phenotype was due to an X-linked mutation?

**ANS:** Cross the singed male to wild-type females and then intercross the offspring. If the singed bristle phenotype is due to an X-linked mutation, approximately half the  $F_2$  males, but none of the  $F_2$  females, will show it.

- 5.3** In grasshoppers, rosy body color is caused by a recessive mutation; the wild-type body color is green. If the gene for body color is on the X chromosome, what kind of progeny would be obtained from a mating between a homozygous rosy female and a hemizygous wild-type male? (In grasshoppers, females are XX and males are XO.)

**ANS:** All the daughters will be green and all the sons will be rosy.

- 5.4** In the mosquito *Anopheles culicifacies*, golden body (*go*) is a recessive X-linked mutation, and brown eyes (*bw*) is a recessive autosomal mutation. A homozygous XX female with golden body is mated to a homozygous XY male with brown eyes. Predict the phenotypes of their  $F_1$  offspring. If the  $F_1$  progeny are intercrossed, what kinds of progeny will appear in the  $F_2$  and in what proportions?

**ANS:** The cross is  $go/go +/+$  female  $\times +/Y bw/bw$  male  $\rightarrow F_1$ :  $go/+ bw/+$  females (wild-type eyes and body) and  $go/Y bw/+$  males (golden body, wild-type eyes). An intercross of the  $F_1$  offspring yields the following  $F_2$  phenotypes in both sexes.

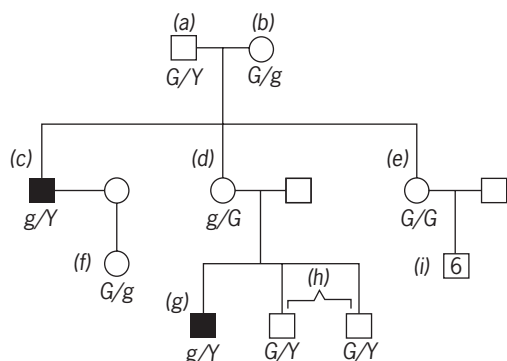
Body	Eyes	Genotype	Proportion
Golden	Brown	$go/go$ or Y	$bw/bw$ $(1/2) \times (1/4)$ $= 1/8$
Golden	Wild-type	$go/go$ or Y	$+/bw$ or + $(1/2) \times (3/4)$ $= 3/8$
Wild-type	Brown	$+/go$ or Y	$bw/bw$ $(1/2) \times (1/4)$ $= 1/8$
Wild-type	Wild-type	$+/go$ or Y	$+/bw$ or + $(1/2) \times (3/4)$ $= 3/8$

14-WC Answers to All Questions and Problems

5.5 What are the sexual phenotypes of the following genotypes in *Drosophila*: XX, XY, XXY, XXX, XO?

**ANS:** XX is female, XY is male, XXY is female, XXX is female (but barely viable), XO is male (but sterile).

5.6 In human beings, a recessive X-linked mutation, *g*, causes green-defective color vision; the wild-type allele, *G*, causes normal color vision. A man (a) and a woman (b), both with normal vision, have three children, all married to people with normal vision: a color-defective son (c), who has a daughter with normal vision (f); a daughter with normal vision (d), who has one color-defective son (g) and two normal sons (h); and a daughter with normal vision (e), who has six normal sons (i). Give the most likely genotypes for the individuals (a–i) in this family.



**ANS:** (a)  $X^G Y$ ; (b)  $X^G X^g$ ; (c)  $X^g Y$ ; (d)  $X^G X^g$ ; (e)  $X^G X^G$ ; (f)  $X^G X^g$ ; (g)  $X^g Y$ ; (h)  $X^G Y$ ; (i)  $X^G Y$

5.7 If both father and son have defective color vision, is it likely that the son inherited the trait from his father?

**ANS:** No. Defective color vision is caused by an X-linked mutation. The son's X chromosome came from his mother, not his father.

5.8 A normal woman, whose father had hemophilia, marries a normal man. What is the chance that their first child will have hemophilia?

**ANS:** The risk for the child is  $P(\text{woman transmits mutant allele}) \times P(\text{child is male}) = (1/2) \times (1/2) = 1/4$ .

5.9 A man with X-linked color blindness marries a woman with no history of color blindness in her family. The daughter of this couple marries a normal man, and their daughter also marries a normal man. What is the chance that this last couple will have a child with color blindness? If this couple has already had a child with color blindness, what is the chance that their next child will be color blind?

**ANS:** The risk for the child is  $P(\text{mother is } C/c) \times P(\text{mother transmits } c) \times P(\text{child is male}) = (1/2) \times (1/2) \times (1/2) = 1/8$ ; if the couple has already had a child with color blindness,  $P(\text{mother is } C/c) = 1$ , and the risk for each subsequent child is  $1/4$ .

5.10 A man who has color blindness and type O blood has children with a woman who has normal color vision and type AB blood. The woman's father had color blindness.

Color blindness is determined by an X-linked gene, and blood type is determined by an autosomal gene.

(a) What are the genotypes of the man and the woman?

(b) What proportion of their children will have color blindness and type B blood?

(c) What proportion of their children will have color blindness and type A blood?

(d) What proportion of their children will be color blind and have type AB blood?

**ANS:** (a) The man is  $X^c Y ii$ ; the woman is  $X^+ X^c I^A I^B$ . (b) Probability color blind =  $1/2$ ; probability type B blood =  $1/2$ ; combined probability =  $(1/2) \times (1/2) = 1/4$ . (c) Probability color blind =  $1/2$ ; probability type A blood =  $1/2$ ; combined probability  $(1/2) \times (1/2) = 1/4$ . (d) 0.

5.11 A *Drosophila* female homozygous for a recessive X-linked mutation that causes vermilion eyes is mated to a wild-type male with red eyes. Among their progeny, all the sons have vermilion eyes, and nearly all the daughters have red eyes; however, a few daughters have vermilion eyes. Explain the origin of these vermilion-eyed daughters.

**ANS:** Each of the rare vermilion daughters must have resulted from the union of an  $X(v) X(v)$  egg with a Y-bearing sperm. The diplo-X eggs must have originated through nondisjunction of the X chromosomes during oogenesis in the mother. However, we cannot determine if the nondisjunction occurred in the first or the second meiotic division.

5.12 In *Drosophila*, vermilion eye color is due to a recessive allele (*v*) located on the X chromosome. Curved wings are due to a recessive allele (*cu*) located on one autosome, and ebony body is due to a recessive allele (*e*) located on another autosome. A vermilion male is mated to a curved, ebony female, and the  $F_1$  males are phenotypically wild-type. If these males were backcrossed to curved, ebony females, what proportion of the  $F_2$  offspring will be wild-type males?

**ANS:**  $P(\text{male}) = 1/2$ ;  $P(\text{male transmits first wild-type autosome}) = 1/2$ ;  $P(\text{male transmits other wild-type autosome}) = 1/2$ ; therefore, combined proportion,  $P(\text{wild-type male}) = 1/8$

5.13 A *Drosophila* female heterozygous for the recessive X-linked mutation *w* (for white eyes) and its wild-type allele  $w^1$  is mated to a wild-type male with red eyes. Among the sons, half have white eyes and half have red eyes. Among the daughters, nearly all have red eyes; however, a few have white eyes. Explain the origin of these white-eyed daughters.

**ANS:** Each of the rare white-eyed daughters must have resulted from the union of an  $X(w) X(w)$  egg with a Y-bearing sperm. The rare diplo-X eggs must have originated through nondisjunction of the X chromosomes during the second meiotic division in the mother.

**5.14** In *Drosophila*, a recessive mutation called *chocolate* (*c*) causes the eyes to be darkly pigmented. The mutant phenotype is indistinguishable from that of an autosomal recessive mutation called *brown* (*bw*). A cross of chocolate-eyed females to homozygous brown males yielded wild-type  $F_1$  females and darkly pigmented  $F_1$  males. If the  $F_1$  flies are intercrossed, what types of progeny are expected, and in what proportions? (Assume that the double mutant combination has the same phenotype as either of the single mutants alone.)

**ANS:** 3/8 wild-type (red), 5/8 brown for both male and female  $F_2$  progeny.

**5.15** Suppose that a mutation occurred in the *SRY* gene on the human Y chromosome, knocking out its ability to produce the testis-determining factor. Predict the phenotype of an individual who carried this mutation and a normal X chromosome.

**ANS:** Female.

**5.16** A woman carries the androgen-insensitivity mutation (*ar*) on one of her X chromosomes; the other X carries the wild-type allele (*AR*). If the woman marries a normal man, what fraction of her children will be phenotypically female? Of these, what fraction will be fertile?

**ANS:** Three-fourths will be phenotypically female (genotypically *ar/AR*, *AR/AR*, or *ar/Y*). Among the females, 2/3 (*ar/AR* and *AR/AR*) will be fertile; the *ar/Y* females will be sterile.

**5.17** Would a human with two X chromosomes and a Y chromosome be male or female?

**ANS:** Male.

**5.18** In *Drosophila*, the gene for *bobbed* bristles (recessive allele *bb*, bobbed bristles; wild-type allele<sup>+</sup>, normal bristles) is located on the X chromosome and on a homologous segment of the Y chromosome. Give the genotypes and phenotypes of the offspring from the following crosses:

(a)  $X^{bb} X^{bb} \times X^{bb} Y^+$ ;

(b)  $X^{bb} X^{bb} \times X^{bb} Y^+$ ;

(c)  $X^+ X^{bb} \times X^+ Y^{bb}$ ;

(d)  $X^+ X^{bb} \times X^{bb} Y^+$ .

**ANS:** (a) 1/2  $X^{bb} X^{bb}$  bobbed females, 1/2  $X^{bb} Y^+$  wild-type males; (b) 1/2  $X^+ X^{bb}$  wild-type females, 1/2  $X^{bb} Y^{bb}$  bobbed males; (c) 1/4  $X^+ X^+$  wild-type females, 1/4  $X^+ X^{bb}$  wild-type females, 1/4  $X^+ Y^{bb}$  wild-type males, 1/4  $X^{bb} Y^{bb}$  bobbed males; (d) 1/4  $X^+ X^{bb}$  wild-type females, 1/4  $X^{bb} X^{bb}$  bobbed females, 1/4  $X^+ Y^+$  wild-type males, 1/4  $X^{bb} Y^+$  wild-type males.

**5.19** Predict the sex of *Drosophila* with the following chromosome compositions (A = haploid set of autosomes):

(a) 4X 4A;

(b) 3X 4A;

(c) 2X 3A;

(d) 1X 3A;

(e) 2X 2A;

(f) 1X 2A.

**ANS:** (a) Female; (b) intersex; (c) intersex; (d) male; (e) female; (f) male.

**5.20** In chickens, the absence of barred feathers is due to a recessive allele. A barred rooster was mated with a nonbarred hen, and all the offspring were barred. These  $F_1$  chickens were intercrossed to produce  $F_2$  progeny, among which all the males were barred; half the females were barred and half were nonbarred. Are these results consistent with the hypothesis that the gene for barred feathers is located on one of the sex chromosomes?

**ANS:** Yes. The gene for feather patterning is on the Z chromosome. If we denote the allele for barred feathers as *B* and the allele for nonbarred feathers as *b*, the crosses are as follows: *B/B* (barred) male  $\times$  *b/W* (nonbarred) female  $\rightarrow$   $F_1$ : *B/b* (barred) males and *B/W* (barred) females. Intercrossing the  $F_1$  produces *B/B* (barred) males, *B/b* (barred) males, *B/W* (barred) females, and *b/W* (nonbarred) females, all in equal proportions.

**5.21** A *Drosophila* male carrying a recessive X-linked mutation for yellow body is mated to a homozygous wild-type female with gray body. The daughters of this mating all have uniformly gray bodies. Why are not their bodies a mosaic of yellow and gray patches?

**ANS:** *Drosophila* does not achieve dosage compensation by inactivating one of the X chromosomes in females.

**5.22** What is the maximum number of Barr bodies in the nuclei of human cells with the following chromosome compositions:

(a) XY;

(b) XX;

(c) XXY;

(d) XXX;

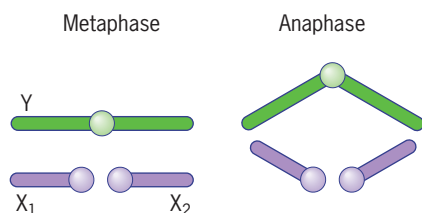
(e) XXXX;

(f) XYY?

**ANS:** a) Zero; (b) one; (c) one; (d) two; (e) three; (f) zero.

**5.23** Males in a certain species of deer have two nonhomologous X chromosomes, denoted  $X_1$  and  $X_2$ , and a Y chromosome. Each X chromosome is about half as large as the Y chromosome, and its centromere is located near one of the ends; the centromere of the Y chromosome is located in the middle. Females in this species have two copies of each of the X chromosomes and lack a Y chromosome. How would you predict the X and Y chromosomes to pair and disjoin during spermatogenesis to produce equal numbers of male- and female-determining sperm?

ANS:



Since the centromere is at the end of each small X chromosome but in the middle of the larger Y, both  $X_1$  and  $X_2$  pair at the centromere of the Y chromosome during metaphase so that the two X chromosomes disjoin together and segregate from the Y chromosome during anaphase.

5.24 A breeder of sun conures (a type of bird) has obtained two true-breeding strains, A and B, which have red eyes instead of the normal brown found in natural populations. In Cross 1, a male from strain A was mated to a female from strain B, and the male and female offspring all had brown eyes. In Cross 2, a female from strain A was mated to a male from strain B, and the male offspring had brown eyes and the female offspring had red eyes. When the  $F_1$  birds from each cross were mated brother to sister, the breeder obtained the following results:

Phenotype	Proportion in $F_2$ of Cross 1	Proportion in $F_2$ of Cross 2
Brown male	6/16	3/16
Red male	3/16	5/16
Brown female	3/16	3/16
Red female	5/16	5/16

Provide a genetic explanation for these results.

ANS: Color is determined by an autosomal gene (alleles  $A$  and  $a$ ) and a sex-linked gene (alleles  $B$  and  $b$ ) on the Z chromosome (females are ZW and males are ZZ) and the recessive alleles are mutually epistatic—that is,  $aa$ ,  $bb$ , or  $bW$  birds have red eyes, and  $A-$   $B-$  or  $A-$   $BW$  birds have brown eyes.

Cross 1

P	Strain A red male $aa BB$	×	Strain B red female $AA bW$
$F_1$	$Aa Bb$ Brown males	×	$Aa BW$ Brown females
$F_2$	$A- Bb$ Brown males (6/16) $aa Bb$ Red males (2/16)		$A- BW$ Brown females (3/16) $A- bW$ Red females (4/16) $aa BW$ Red females (1/16)

Cross 2

P	Strain A red female $aa BW$	×	Strain B red male $AA bb$
$F_1$	$Aa Bb$ Brown males	×	$Aa bW$ Red females
$F_2$	$A- Bb$ Brown males (3/16) $A- bb$ Red males (3/16) $aa b-$ Red males 2/16		$A- bW$ Brown females (3/16) $A- bW$ Red females (3/16) $aa -W$ Red females (2/16)

5.25 In 1908, F. M. Durham and D. C. E. Marryat reported the results of breeding experiments with canaries. Cinnamon canaries have pink eyes when they first hatch, whereas green canaries have black eyes. Durham and Marryat crossed cinnamon females with green males and observed that all the  $F_1$  progeny had black eyes, just like those of the green strain. When the  $F_1$  males were crossed to green females, all the male progeny had black eyes, whereas all the female progeny had either black or pink eyes, in about equal proportions. When the  $F_1$  males were crossed to cinnamon females, four classes of progeny were obtained: females with black eyes, females with pink eyes, males with black eyes, and males with pink eyes—all in approximately equal proportions. Propose an explanation for these findings.

ANS: Eye color in canaries is due to a gene on the Z chromosome, which is present in two copies in males and one copy in females. The allele for pink color at hatching ( $p$ ) is recessive to the allele for black color at hatching ( $P$ ). There is no eye color gene on the other sex chromosome ( $W$ ), which is present in one copy in females and absent in males. The parental birds were genotypically  $p/W$  (cinnamon females) and  $P/P$  (green males). Their  $F_1$  sons were genotypically  $p/P$  (with black eyes at hatching). When these sons were crossed to green females (genotype  $P/W$ ), they produced  $F_2$  progeny that sorted into three categories: males with black eyes at hatching ( $P/-$ , half the total progeny), females with black eyes at hatching ( $P/W$ , a fourth of the total progeny), and females with pink eyes at hatching ( $p/W$ , a fourth of the total progeny). When these sons were crossed to cinnamon females (genotype  $p/W$ ), they produced  $F_2$  progeny that sorted into four equally frequent categories: males with black eyes at hatching (genotype  $P/p$ ), males with pink eyes at hatching (genotype  $p/p$ ), females with black eyes at hatching (genotype  $P/W$ ), and females with pink eyes at hatching (genotype  $p/W$ ).



## CHAPTER 6

**6.1** In the human karyotype, the X chromosome is approximately the same size as seven of the autosomes (the so-called C group of chromosomes). What procedure could be used to distinguish the X chromosome from the other members of this group?

**ANS:** Use one of the banding techniques.

**6.2** In humans, a cytologically abnormal chromosome 22, called the “Philadelphia” chromosome because of the city in which it was discovered, is associated with chronic leukemia. This chromosome is missing part of its long arm. How would you denote the karyotype of an individual who had 46 chromosomes in his somatic cells, including one normal 22 and one Philadelphia chromosome?

**ANS:** 46, XX, del(22)(q) or 46, XY, del(22)(q), depending on the sex chromosome constitution.

**6.3** During meiosis, why do some tetraploids behave more regularly than triploids?

**ANS:** In allotetraploids, each member of the different sets of chromosomes can pair with a homologous partner during prophase I and then disjoin during anaphase I. In triploids, disjunction is irregular because homologous chromosomes associate during prophase I either by forming bivalents and univalents or by forming trivalents.

**6.4** The following table presents chromosome data on four species of plants and their  $F_1$  hybrids:

## Meiosis I Metaphase

Species or $F_1$ Hybrid	Root Tip Chromosome Number	Number of Bivalents	Number of Univalents
A	20	10	0
B	20	10	0
C	10	5	0
D	10	5	0
A × B	20	0	20
A × C	15	5	5
A × D	15	5	5
C × D	10	0	10

(a) Deduce the chromosomal origin of species A.

(b) How many bivalents and univalents would you expect to observe at meiotic metaphase I in a hybrid between species C and species B?

(c) How many bivalents and univalents would you expect to observe at meiotic metaphase I in a hybrid between species D and species B?

**ANS:** (a) Species A is an allotetraploid with a genome from each of species C and species D; (b) 0 bivalents and 15 univalents; (c) 0 bivalents and 15 univalents.

**6.5** A plant species A, which has seven chromosomes in its gametes, was crossed with a related species B, which has nine. The hybrids were sterile, and microscopic observation of their pollen mother cells showed no chromosome pairing. A section from one of the hybrids that grew vigorously was propagated vegetatively, producing a plant with 32 chromosomes in its somatic cells. This plant was fertile. Explain.

**ANS:** The fertile plant is an allotetraploid with 7 pairs of chromosomes from species A and 9 pairs of chromosomes from species B; the total number of chromosomes is  $(2 \times 7) + (2 \times 9) = 32$ .

**6.6** A plant species X with  $n = 5$  was crossed with a related species Y with  $n = 7$ . The  $F_1$  hybrid produced only a few pollen grains, which were used to fertilize the ovules of species Y. A few plants were produced from this cross, and all had 19 chromosomes. Following self-fertilization, the  $F_1$  hybrids produced a few  $F_2$  plants, each with 24 chromosomes. These plants were phenotypically different from either of the original species and were highly fertile. Explain the sequence of events that produced these fertile  $F_2$  hybrids.

**ANS:** The  $F_1$  hybrid had 5 chromosomes from species X and 7 chromosomes from species Y, for a total of 12. When this hybrid was backcrossed to species Y, the few progeny that were produced had  $5 + 7 = 12$  chromosomes from the hybrid and 7 from species Y, for a total of 19. This hybrid was therefore a triploid. Upon self-fertilization, a few  $F_2$  plants were formed, each with 24 chromosomes. Presumably the chromosomes in these plants consisted of  $2 \times 5 = 10$  from species X and  $2 \times 7 = 14$  from species Y. These vigorous and fertile  $F_2$  plants were therefore allotetraploids.

**6.7** Identify the sexual phenotypes of the following genotypes in human beings: XX, XY, XO, XXX, XXY, XYY.

**ANS:** XX is female, XY is male, XO is female (but sterile), XXX is female, XXY is male (but sterile), and XYY is male.

**6.8** If nondisjunction of chromosome 21 occurs in the division of a secondary oocyte in a human female, what is the chance that a mature egg derived from this division will receive two number 21 chromosomes?

**ANS:** 1/2

**6.9** A *Drosophila* female homozygous for a recessive X-linked mutation causing yellow body was crossed to a wild-type male. Among the progeny, one fly had sectors of yellow pigment in an otherwise gray body. These yellow sectors were distinctly male, whereas the gray areas were female. Explain the peculiar phenotype of this fly.

**ANS:** The fly is a gynandromorph, that is, a sexual mosaic. The yellow tissue is  $X(y)/O$  and the gray tissue is  $X(y)/X(+)$ . This mosaicism must have arisen through loss of the X chromosome that carried the wild-type allele, presumably during one of the early embryonic cleavage divisions.

**6.10** The *Drosophila* fourth chromosome is so small that flies monosomic or trisomic for it survive and are fertile. Several genes, including *eyeless* (*ey*), have been located on this chromosome. If a cytologically normal fly homozygous for a recessive eyeless mutation is crossed to a fly monosomic for a wild-type fourth chromosome, what kinds of progeny will be produced, and in what proportions?

**ANS:** Approximately half the progeny should be disomic  $ey/+$  and half should be monosomic  $ey/O$ . The disomic progeny will be wild-type, and the monosomic progeny will be eyeless.

**6.11** A woman with X-linked color blindness and Turner syndrome had a color-blind father and a normal mother. In which of her parents did nondisjunction of the sex chromosomes occur?

**ANS:** Nondisjunction must have occurred in the mother. The color blind woman with Turner syndrome was produced by the union of an X-bearing sperm, which carried the mutant allele for color blindness, and a nullo-X egg.

**6.12** In humans, Hunter syndrome is known to be an X-linked trait with complete penetrance. In family A, two phenotypically normal parents have produced a normal son, a *daughter* with Hunter and Turner syndromes, and a son with Hunter syndrome. In family B, two phenotypically normal parents have produced two phenotypically normal daughters and a *son* with Hunter and Klinefelter syndromes. In family C, two phenotypically normal parents have produced a phenotypically normal daughter, a *daughter* with Hunter syndrome, and a son with Hunter syndrome. For each family, explain the origin of the child indicated in italics.

**ANS:** The daughter with Turner and Hunter syndromes in family A must have received her single X chromosome from her mother, who is heterozygous for the mutation causing Hunter syndrome. The daughter did not receive a sex chromosome from her father because sex chromosome nondisjunction must have occurred during meiosis in his germline. The son with Klinefelter syndrome in family B is karyotypically XXY, and both of his X chromosomes carry the mutant allele for Hunter syndrome. This individual must have received two mutant X chromosomes from his heterozygous mother due to X chromosome nondisjunction during the second meiotic division in her germline. The daughter with Hunter syndrome in family C is karyotypically XX, and both of her X chromosomes carry the mutant allele for Hunter syndrome. This individual received the two mutant X

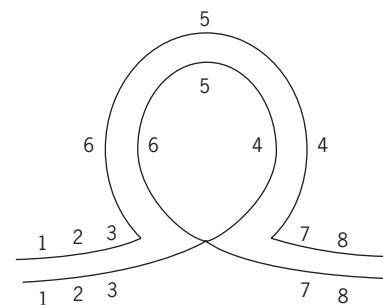
chromosomes from her heterozygous mother through nondisjunction during the second meiotic division in the mother's germline. Furthermore, because the daughter did not receive a sex chromosome from her father, sex chromosome nondisjunction must have occurred during meiosis in his germline too.

**6.13** Although XYY men are phenotypically normal, would they be expected to produce more children with sex chromosome abnormalities than XY men? Explain.

**ANS:** XYY men would produce more children with sex chromosome abnormalities because their three sex chromosomes will disjoin irregularly during meiosis. This irregular disjunction will produce a variety of aneuploid gametes, including the XY, YY, XYY, and nullo sex chromosome constitutions.

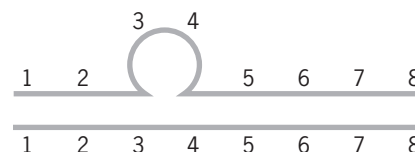
**6.14** In a *Drosophila* salivary chromosome, the bands have a sequence of 1 2 3 4 5 6 7 8. The homologue with which this chromosome is synapsed has a sequence of 1 2 3 6 5 4 7 8. What kind of chromosome change has occurred? Draw the synapsed chromosomes.

**ANS:** The animal is heterozygous for an inversion:

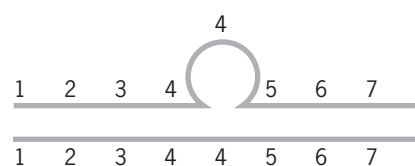


**6.15** Other chromosomes have sequences as follows: (a) 1 2 5 6 7 8; (b) 1 2 3 4 4 5 6 7 8; (c) 1 2 3 4 5 8 7 6. What kind of chromosome change is present in each? Illustrate how these chromosomes would pair with a chromosome whose sequence is 1 2 3 4 5 6 7 8.

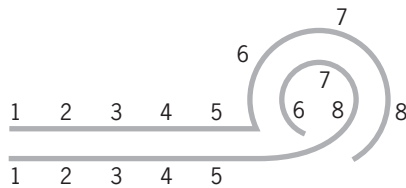
**ANS:** (a) Deletion:



(b) Duplication:

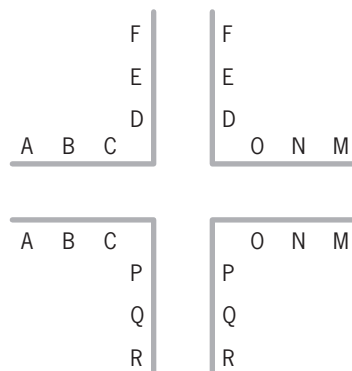


(c) A terminal inversion:



- 6.16** In plants translocation heterozygotes display about 50 percent pollen abortion. Why?
- ANS:** In translocation heterozygotes, only alternate segregation leads to euploid gametes, and the frequency of alternate segregation is typically around 50 percent.
- 6.17** One chromosome in a plant has the sequence A B C D E F, and another has the sequence M N O P Q R. A reciprocal translocation between these chromosomes produced the following arrangement: A B C P Q R on one chromosome and M N O D E F on the other. Illustrate how these translocated chromosomes would pair with their normal counterparts in a heterozygous individual during meiosis.

**ANS:**



- 6.18** In *Drosophila*, the genes *bw* and *st* are located on chromosomes 2 and 3, respectively. Flies homozygous for *bw* mutations have brown eyes, flies homozygous for *st* mutations have scarlet eyes, and flies homozygous for *bw* and *st* mutations have white eyes. Doubly heterozygous males were mated individually to homozygous *bw*; *st* females. All but one of the matings produced four classes of progeny: wild-type, and brown-, scarlet- and white-eyed. The single exception produced only wild-type and white-eyed progeny. Explain the nature of this exception.
- ANS:** The exceptional male, whose genotype is *bw*/+ *st*/+, is heterozygous for a translocation between chromosomes 2 and 3. It is not possible to determine whether the translocation is between the two mutant chromosomes or between the two wild-type chromosomes, that is, whether it is T(*bw*; *st*) or T(+; +); however, it clearly is

not between a mutant chromosome and a wild-type chromosome, that is, T(*bw*; +) or T(+; *st*). If it were, the progeny would be either brown or scarlet, not either wild-type or white.

- 6.19** A phenotypically normal boy has 45 chromosomes, but his sister, who has Down syndrome, has 46. Suggest an explanation for this paradox.
- ANS:** The boy carries a translocation between chromosome 21 and another chromosome, say chromosome 14. He also carries a normal chromosome 21 and a normal chromosome 14. The boy's sister carries the translocation, one normal chromosome 14, and two normal copies of chromosome 21.
- 6.20** Distinguish between a compound chromosome and a Robertsonian translocation.

**ANS:** A compound chromosome is composed of segments from the same pair of chromosomes, as when two X chromosomes become attached to each other. A Robertsonian translocation involves a fusion of segments from two different pairs of chromosomes. These segments fuse at or near the centromeres, usually with the loss of the short arms of each of the participating chromosomes.

- 6.21** A yellow-bodied *Drosophila* female with attached-X chromosomes was crossed to a white-eyed male. Both of the parental phenotypes are caused by X-linked recessive mutations. Predict the phenotypes of the progeny.

**ANS:** All the daughters will be yellow-bodied and all the sons will be white-eyed.

- 6.22** A man has attached chromosomes 21. If his wife is cytologically normal, what is the chance their first child will have Down syndrome?

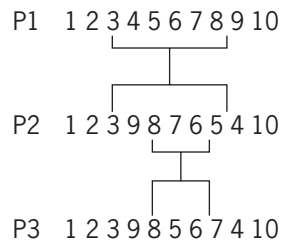
**ANS:** Zygotes produced by this couple will be either trisomic or monosomic for chromosome 21. Thus, 100 percent of their viable children will develop Down syndrome.

- 6.23** Analysis of the polytene chromosomes of three populations of *Drosophila* has revealed three different banding sequences in a region of the second chromosome:

Population	Banding Sequence
P1	1 2 3 4 5 6 7 8 9 10
P2	1 2 3 9 8 7 6 5 4 10
P3	1 2 3 9 8 5 6 7 4 10

Explain the evolutionary relationships among these populations.

**ANS:** The three populations are related by a series of inversions:



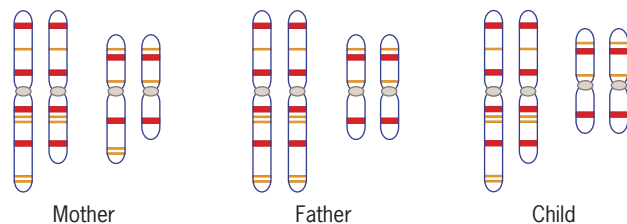
**6.24** Each of six populations of *Drosophila* in different geographic regions had a specific arrangement of bands in one of the large autosomes:

- 12345678
- 12263478
- 15432678
- 14322678
- 16223478
- 154322678

Assume that arrangement (a) is the original one. In what order did the other arrangements most likely arise, and what type of chromosomal aberration is responsible for each change?

**ANS:** Arrangement (a) produced (c) by inversion of segment 2345; (c) produced (f) by a duplication of band 2; (f) produced (d) by a deletion of band 5; (d) produced (e) by inversion of segment 43226; (e) produced (b) by inversion of segment 622.

**6.25** The following diagram shows two pairs of chromosomes in the karyotypes of a man, a woman, and their child. The man and the woman are phenotypically normal, but the child (a boy) suffers from a syndrome of abnormalities, including poor motor control and severe mental impairment. What is the genetic basis of the child's abnormal phenotype? Is the child hyperploid or hypoploid for a segment in one of his chromosomes?



**ANS:** The mother is heterozygous for a reciprocal translocation between the long arms of the large and small chromosomes; a piece from the long arm of the large chromosome has been broken off and attached to the long arm of the short chromosome. The child has inherited the rearranged large chromosome and the normal small chromosome from the mother. Thus, because the rearranged large chromosome is deficient for some of its genes, the child is hypoploid.

**6.26** A male mouse that is heterozygous for a reciprocal translocation between the X chromosome and an autosome is crossed to a female mouse with a normal karyotype. The autosome involved in the translocation carries a gene responsible for coloration of the fur. The allele on the male's translocated autosome is wild-type, and the allele on its nontranslocated autosome is mutant; however, because the wild-type allele is dominant to the mutant allele, the male's fur is wild-type (dark in color). The female mouse has light color in her fur because she is homozygous for the mutant allele of the color-determining gene. When the offspring of the cross are examined, all the males have light fur and all the females have patches of light and dark fur. Explain these peculiar results.

**ANS:** The phenotype in the female offspring is mosaic because one of the X chromosomes is inactivated in each of their cells. If the translocated X is inactivated, the autosome attached to it could also be partially inactivated by a spreading of the inactivation process across the translocation breakpoint. This spreading could therefore inactivate the color-determining gene on the translocated autosome and cause patches of tissue to be phenotypically mutant.

**6.27** In *Drosophila*, the autosomal genes *cinnabar* (*cn*) and *brown* (*bw*) control the production of brown and red eye pigments, respectively. Flies homozygous for *cinnabar* mutations have bright red eyes, flies homozygous for *brown* mutations have brown eyes, and flies homozygous for mutations in both of these genes have white eyes. A male homozygous for mutations in the *cn* and *bw* genes has bright red eyes because a small duplication that carries the wild-type allele of *bw* ( $bw^+$ ) is attached to the Y chromosome. If this male is mated to a karyotypically normal female that is homozygous for the *cn* and *bw* mutations, what types of progeny will be produced?

**ANS:** The sons will have bright red eyes because they will inherit the Y chromosome with the  $bw^+$  allele from their father. The daughters will have white eyes because they will inherit an X chromosome from their father.

**6.28** In *Drosophila*, vestigial wing (*vg*), hairy body (*b*), and eyeless (*ey*) are recessive mutations on chromosomes 2, 3, and 4, respectively. Wild-type males that had been irradiated with X rays were crossed to triply homozygous recessive females. The  $F_1$  males (all phenotypically wild-type) were then testcrossed to triply homozygous recessive females. Most of the  $F_1$  males produced eight classes of progeny in approximately equal proportions, as would be expected if the *vg*, *b*, and *ey* genes assort independently. However, one  $F_1$  male produced only four classes of offspring, each approximately one-fourth of the total: (1) wild-type, (2) eyeless, (3) vestigial, hairy, and (4) vestigial, hairy, eyeless. What kind of chromosome aberration did the exceptional  $F_1$  male carry, and which chromosomes were involved?

**ANS:** A reciprocal translocation between chromosomes 2 and 3. One translocated chromosome carries the wild-type alleles of *vg* and *b* on chromosomes 2 and 3, respectively, and the other carries the recessive mutant alleles of these genes. The chromosome that carries the *ey* gene (chromosome 4) is not involved in the rearrangement.

**6.29** Cytological examination of the sex chromosomes in a man has revealed that he carries an insertional translocation. A small segment has been deleted from the Y chromosome and inserted into the short arm of the X chromosome; this segment contains the gene responsible for male differentiation (*SRY*). If this man marries a karyotypically normal woman, what types of progeny will the couple produce?

**ANS:** XX zygotes will develop into males because one of their X chromosomes carries the *SRY* gene that was translocated from the Y chromosome. XY zygotes will develop into females because their Y chromosome has lost the *SRY* gene.

### CHAPTER 7

**7.1** Mendel did not know of the existence of chromosomes. Had he known, what change might he have made in his Principle of Independent Assortment?

**ANS:** If Mendel had known of the existence of chromosomes, he would have realized that the number of factors determining traits exceeds the number of chromosomes, and he would have concluded that some factors must be linked on the same chromosome. Thus, Mendel would have revised the Principle of Independent Assortment to say that factors on different chromosomes (or far apart on the same chromosome) are inherited independently.

**7.2** From a cross between individuals with the genotypes *Cc Dd Ee* × *cc dd ee*, 1000 offspring were produced. The class that was *C- D- ee* included 351 individuals. Are the genes *c*, *d*, and *e* on the same or different chromosomes? Explain.

**ANS:** The class represented by 351 offspring indicates that at least two of the three genes are linked.

**7.3** If *a* is linked to *b*, and *b* to *c*, and *c* to *d*, does it follow that a recombination experiment would detect linkage between *a* and *d*? Explain.

**ANS:** No. The genes *a* and *d* could be very far apart on the same chromosome—so far apart that they recombine freely, that is, 50 percent of the time.

**7.4** Mice have 19 autosomes in their genome, each about the same size. If two autosomal genes are chosen randomly, what is the chance that they will be on the same chromosome?

**ANS:** 1/19

**7.5** Genes on different chromosomes recombine with a frequency of 50 percent. Is it possible for two genes on the same chromosome to recombine with this frequency?

**ANS:** Yes, if they are very far apart.

**7.6** If two loci are 10 cM apart, what proportion of the cells in prophase of the first meiotic division will contain a single crossover in the region between them?

**ANS:** 20%

**7.7** Genes *a* and *b* are 20 cM apart. An *a<sup>+</sup> b<sup>+</sup>/a<sup>+</sup> b<sup>+</sup>* individual was mated with an *a b/a b* individual.

(a) Diagram the cross and show the gametes produced by each parent and the genotype of the F<sub>1</sub>.

(b) What gametes can the F<sub>1</sub> produce, and in what proportions?

(c) If the F<sub>1</sub> was crossed to *a b/a b* individuals, what offspring would be expected, and in what proportions?

(d) Is this an example of the coupling or repulsion linkage phase?

(e) If the F<sub>1</sub> were intercrossed, what offspring would be expected, and in what proportions?

**ANS:** (a) Cross: *a<sup>+</sup> b<sup>+</sup>/a<sup>+</sup> b<sup>+</sup>* × *a b/a b*. Gametes: *a<sup>+</sup> b<sup>+</sup>* from one parent, *a b* from the other. F<sub>1</sub>: *a<sup>+</sup> b<sup>+</sup>/a b*

(b) 40% *a<sup>+</sup> b<sup>+</sup>*, 40% *a b*, 10% *a<sup>+</sup> b*, 10% *a b<sup>+</sup>*

(c) F<sub>2</sub> from testcross: 40% *a<sup>+</sup> b<sup>+</sup>/a b*, 40% *a b/a b*, 10% *a<sup>+</sup> b/a b*, 10% *a b<sup>+</sup>/a b*

(d) Coupling linkage phase

(e) F<sub>2</sub> from intercross:

		Sperm			
		40% <i>a<sup>+</sup> b<sup>+</sup></i>	40% <i>a b</i>	10% <i>a<sup>+</sup> b</i>	10% <i>a b<sup>+</sup></i>
Eggs	40% <i>a<sup>+</sup> b<sup>+</sup></i>	16% <i>a<sup>+</sup> b<sup>+</sup>/a<sup>+</sup> b<sup>+</sup></i>	16% <i>a<sup>+</sup> b<sup>+</sup>/a b</i>	4% <i>a<sup>+</sup> b<sup>+</sup>/a<sup>+</sup> b</i>	4% <i>a<sup>+</sup> b<sup>+</sup>/a b<sup>+</sup></i>
	40% <i>a b</i>	16% <i>a b/a<sup>+</sup> b<sup>+</sup></i>	16% <i>a b/a b</i>	4% <i>a b/a<sup>+</sup> b</i>	4% <i>a b/a b<sup>+</sup></i>
	10% <i>a<sup>+</sup> b</i>	4% <i>a<sup>+</sup> b/a<sup>+</sup> b<sup>+</sup></i>	4% <i>a<sup>+</sup> b/a b</i>	1% <i>a<sup>+</sup> b/a<sup>+</sup> b</i>	1% <i>a<sup>+</sup> b/a b<sup>+</sup></i>
	10% <i>a b<sup>+</sup></i>	4% <i>a b<sup>+</sup>/a<sup>+</sup> b<sup>+</sup></i>	4% <i>a b<sup>+</sup>/a b</i>	1% <i>a b<sup>+</sup>/a<sup>+</sup> b</i>	1% <i>a b<sup>+</sup>/a b<sup>+</sup></i>

#### Summary of phenotypes:

<i>a<sup>+</sup></i> and <i>b<sup>+</sup></i>	66%
<i>a<sup>+</sup></i> and <i>b</i>	9%
<i>a</i> and <i>b<sup>+</sup></i>	9%
<i>a</i> and <i>b</i>	16%

**7.8** Answer questions (a)–(e) in the preceding problem under the assumption that the original cross was *a<sup>+</sup> b/a<sup>+</sup> b* × *a b<sup>+</sup>/a b<sup>+</sup>*.

**ANS:** (a) Cross: *a<sup>+</sup> b/a<sup>+</sup> b* × *a b<sup>+</sup>/a b<sup>+</sup>*. Gametes: *a<sup>+</sup> b* from one parent, *a b<sup>+</sup>* from the other F<sub>1</sub>: *a<sup>+</sup> b/a b<sup>+</sup>*

(b) 40% *a<sup>+</sup> b*, 40% *a b<sup>+</sup>*, 10% *a<sup>+</sup> b<sup>+</sup>*, 10% *a b*

(c)  $F_2$  from testcross: 40%  $a^+ b/a b$ , 40%  $a b^+/a b$ , 10%  $a^+ b^+/a b$ , 10%  $a b/a b$

(d) Repulsion linkage phase

(e)  $F_2$  from intercross:

Eggs	Sperm			
	40% $a^+ b$	40% $a b^+$	10% $a^+ b^+$	10% $a b$
40% $a^+ b$	16% $a^+ b/a^+ b$	16% $a^+ b/a b^+$	4% $a^+ b^+/a^+ b^+$	4% $a^+ b/a b$
40% $a b^+$	16% $a b^+/a^+ b$	16% $a b^+/a b^+$	4% $a b^+/a^+ b^+$	4% $a b^+/a b$
10% $a^+ b^+$	4% $a^+ b^+/a^+ b$	4% $a^+ b^+/a b^+$	1% $a^+ b^+/a^+ b^+$	1% $a^+ b^+/a b$
10% $a b$	4% $a b/a^+ b$	4% $a b/a b^+$	1% $a b/a^+ b^+$	1% $a b/a b$

**Summary of phenotypes:**

$a^+$ and $b^+$	51%
$a^+$ and $b$	24%
$a$ and $b^+$	24%
$a$ and $b$	1%

7.9 If the recombination frequency in the previous two problems were 40 percent instead of 20 percent, what change would occur in the proportions of gametes and testcross progeny?

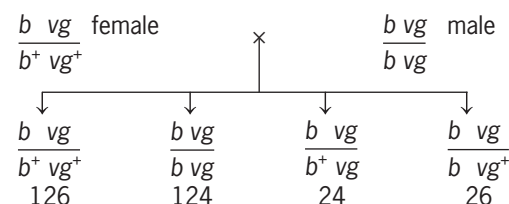
ANS: Coupling heterozygotes  $a^+ b^+/a b$  would produce the following gametes: 30%  $a^+ b^+$ , 30%  $a b$ , 20%  $a^+ b$ , 20%  $a b^+$ ; repulsion heterozygotes  $a^+ b/a b^+$  would produce the following gametes: 30%  $a^+ b$ , 30%  $a b^+$ , 20%  $a^+ b^+$ , 20%  $a b$ . In each case, the frequencies of the testcross progeny would correspond to the frequencies of the gametes.

7.10 A homozygous variety of maize with red leaves and normal seeds was crossed with another homozygous variety with green leaves and tassel seeds. The hybrids were then backcrossed to the green, tassel-seeded variety, and the following offspring were obtained: red, normal 124; red, tassel 126; green, normal 125; green, tassel 123. Are the genes for plant color and seed type linked? Explain.

ANS: No. The leaf color and tassel seed traits assort independently.

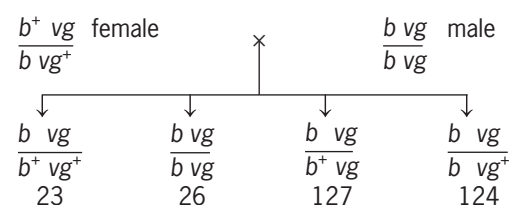
7.11 A phenotypically wild-type female fruit fly that was heterozygous for genes controlling body color and wing length was crossed to a homozygous mutant male with black body (allele  $b$ ) and vestigial wings (allele  $vg$ ). The cross produced the following progeny: gray body, normal wings 126; gray body, vestigial wings 24; black body, normal wings 26; black body, vestigial wings 124. Do these data indicate linkage between the genes for body color and wing length? What is the frequency of recombination? Diagram the cross, showing the arrangement of the genetic markers on the chromosomes.

ANS: Yes. Recombination frequency =  $(24 + 26)/(126 + 24 + 26 + 124) = 0.167$ . Cross:



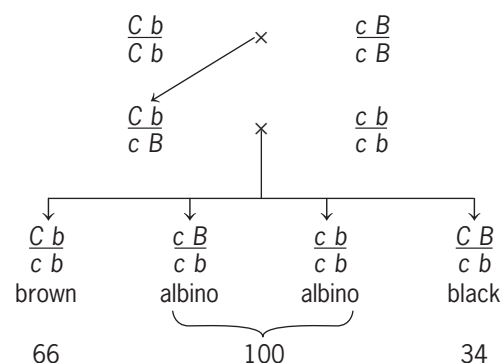
7.12 Another phenotypically wild-type female fruit fly heterozygous for the two genes mentioned in the previous problem was crossed to a homozygous black, vestigial male. The cross produced the following progeny: gray body, normal wings 23; gray body, vestigial wings 127; black body, normal wings 124; black body, vestigial wings 26. Do these data indicate linkage? What is the frequency of recombination? Diagram the cross, showing the arrangement of the genetic markers on the chromosomes.

ANS: Yes. Recombination frequency =  $(23 + 26)/(23 + 127 + 124 + 26) = 0.163$ . Cross:



7.13 In rabbits, the dominant allele  $C$  is required for colored fur; the recessive allele  $c$  makes the fur colorless (albino). In the presence of at least one  $C$  allele, another gene determines whether the fur is black ( $B$ , dominant) or brown ( $b$ , recessive). A homozygous strain of brown rabbits was crossed with a homozygous strain of albinos. The  $F_1$  were then crossed to homozygous double recessive rabbits, yielding the following results: black 34; brown 66; albino 100. Are the genes  $b$  and  $c$  linked? What is the frequency of recombination? Diagram the crosses, showing the arrangement of the genetic markers on the chromosomes.

ANS: Yes. Recombination frequency is estimated by the frequency of black offspring among the colored offspring:  $34/(66 + 34) = 0.34$ . Cross:



**7.14** In tomatoes, tall vine ( $D$ ) is dominant over dwarf ( $d$ ), and spherical fruit shape ( $P$ ) is dominant over pear shape ( $p$ ). The genes for vine height and fruit shape are linked with 20 percent recombination between them. One tall plant (I) with spherical fruit was crossed with a dwarf, pear-fruited plant. The cross produced the following results: tall, spherical 81; dwarf, pear 79; tall, pear 22; dwarf spherical 17. Another tall plant with spherical fruit (II) was crossed with the dwarf, pear-fruited plant, and the following results were obtained: tall, pear 21; dwarf, spherical 18; tall, spherical 5; dwarf, pear 4. Diagram these two crosses, showing the genetic markers on the chromosomes. If the two tall plants with spherical fruit were crossed with each other, that is,  $I \times II$ , what phenotypic classes would you expect from the cross, and in what proportions?

**ANS:** Plant I has the genotype  $D P/d p$ , and when crossed to a  $d p/d p$  plant produces four classes of progeny:

$\underline{D P}$	$\underline{d p}$	$\underline{D p}$	$\underline{d P}$
$d p$	$d p$	$d p$	$d p$
81	79	22	17

Plant II has the genotype  $D p/d P$ , and when crossed to a  $d p/d p$  plant produces four classes of progeny:

$\underline{D p}$	$\underline{d P}$	$\underline{D P}$	$\underline{d p}$
$d p$	$d p$	$d p$	$d p$
21	18	5	4

If the two plants are crossed ( $D P/d p \times D p/d P$ ), the phenotypes of the offspring can be predicted from the following table.

		Gametes from plant I			
		$D p$ 0.40	$d p$ 0.40	$D p$ 0.10	$d P$ 0.10
Gametes from plant II	$D p$ 0.40	$D p/D P$ 0.16	$D p/d p$ 0.16	$D p/D p$ 0.04	$D p/d P$ 0.04
	$d P$ 0.40	$d P/D P$ 0.16	$d P/d p$ 0.16	$d P/D p$ 0.04	$d P/d P$ 0.04
	$D P$ 0.10	$D P/D P$ 0.04	$D P/d p$ 0.04	$D P/D p$ 0.01	$D P/d P$ 0.01
	$d p$ 0.10	$d p/D P$ 0.04	$d p/d p$ 0.04	$d p/D p$ 0.01	$d p/d P$ 0.01

**Summary of phenotypes:**

Tall, spherical	0.54
Tall, pear	0.21
Dwarf, spherical	0.21
Dwarf, pear	0.04

**7.15** In *Drosophila*, the genes  $sr$  (*stripe* thorax) and  $e$  (*ebony* body) are located at 62 and 70 cM, respectively, from the left end of chromosome 3. A striped female

homozygous for  $e^+$  was mated with an ebony male homozygous for  $sr^+$ . All the offspring were phenotypically wild-type (gray body and unstriped).

(a) What kind of gametes will be produced by the  $F_1$  females, and in what proportions?

(b) What kind of gametes will be produced by the  $F_1$  males, and in what proportions?

(c) If the  $F_1$  females are mated with striped, ebony males, what offspring are expected, and in what proportions?

(d) If the  $F_1$  males and females are intercrossed, what offspring would you expect from this intercross, and in what proportions?

**ANS:** (a) The  $F_1$  females, which are  $sr e^+/sr^+ e$ , produce four types of gametes: 46%  $sr e^+$ , 46%  $sr^+ e$ , 4%  $sr e$ , 4%  $sr^+ e^+$ . (b) The  $F_1$  males, which have the same genotype as the  $F_1$  females, produce two types of gametes: 50%  $sr e^+$ , 50%  $sr^+ e$ ; remember, there is no crossing over in *Drosophila* males. (c) 46% striped, gray; 46% unstriped, ebony; 4% striped, ebony; 4% unstriped, gray. (d) The offspring from the intercross can be obtained from the following table.

		Sperm	
		$sr e^+$ 0.50	$sr^+ e$ 0.50
Eggs	$sr e^+$ 0.46	$sr e^+/sr e^+$ 0.23	$sr e^+/sr^+ e$ 0.23
	$sr^+ e$ 0.46	$sr^+ e/sr e^+$ 0.23	$sr^+ e/sr^+ e$ 0.23
	$sr e$ 0.04	$sr e/sr e^+$ 0.002	$sr e/sr^+ e$ 0.002
	$sr^+ e^+$ 0.04	$sr^+ e^+/sr e^+$ 0.002	$sr^+ e^+/sr^+ e$ 0.002

**Summary of phenotypes:**

Striped, gray	0.25
Unstriped, gray	0.50
Striped, ebony	0
Unstriped, ebony	0.25

**7.16** In *Drosophila*, genes  $a$  and  $b$  are located at positions 22.0 and 42.0 on chromosome 2, and genes  $c$  and  $d$  are located at positions 10.0 and 25.0 on chromosome 3. A fly homozygous for the wild-type alleles of these four genes was crossed with a fly homozygous for the recessive alleles, and the  $F_1$  daughters were backcrossed to their quadruply recessive fathers. What offspring would you expect from this backcross, and in what proportions?

**ANS:** Because the two chromosomes assort independently, the genetic makeup of the gametes (and, therefore, of the backcross progeny) can be obtained from the following table.

		Chromosome 3 in gametes			
		<i>c d</i> 0.425	<i>c<sup>+</sup> d<sup>+</sup></i> 0.425	<i>c d<sup>+</sup></i> 0.075	<i>c<sup>+</sup> d</i> 0.075
Chromosome 2 in gametes	<i>a b</i> 0.40	<i>a b c d</i> 0.17	<i>a b c<sup>+</sup> d<sup>+</sup></i> 0.17	<i>a b c d<sup>+</sup></i> 0.03	<i>a b c<sup>+</sup> d</i> 0.03
	<i>a<sup>+</sup> b<sup>+</sup></i> 0.40	<i>a<sup>+</sup> b<sup>+</sup> c d</i> 0.17	<i>a<sup>+</sup> b<sup>+</sup> c<sup>+</sup> d<sup>+</sup></i> 0.17	<i>a<sup>+</sup> b<sup>+</sup> c d<sup>+</sup></i> 0.03	<i>a<sup>+</sup> b<sup>+</sup> c<sup>+</sup> d</i> 0.03
	<i>a<sup>+</sup> b</i> 0.10	<i>a<sup>+</sup> b c d</i> 0.0425	<i>a<sup>+</sup> b<sup>+</sup> c<sup>+</sup> d<sup>+</sup></i> 0.0425	<i>a<sup>+</sup> b c d<sup>+</sup></i> 0.0075	<i>a<sup>+</sup> b c<sup>+</sup> d</i> 0.0075
	<i>a b<sup>+</sup></i> 0.10	<i>a b<sup>+</sup> c d</i> 0.0425	<i>a b<sup>+</sup> c<sup>+</sup> d<sup>+</sup></i> 0.0425	<i>a b<sup>+</sup> c d<sup>+</sup></i> 0.0075	<i>a b<sup>+</sup> c<sup>+</sup> d</i> 0.0075

7.17 The *Drosophila* genes *vg* (vestigial wings) and *cn* (cinnabar eyes) are located at 67.0 and 57.0, respectively, on chromosome 2. A female from a homozygous strain of vestigial flies was crossed with a male from a homozygous strain of cinnabar flies. The F<sub>1</sub> hybrids were phenotypically wild-type (long wings and dark red eyes).

(a) How many different kinds of gametes could the F<sub>1</sub> females produce, and in what proportions?

(b) If these females are mated with cinnabar, vestigial males, what kinds of progeny would you expect, and in what proportions?

ANS: (a) The F<sub>1</sub> females, which are *cn vg<sup>+</sup>/cn<sup>+</sup> vg*, produce four types of gametes: 45% *cn vg<sup>+</sup>*, 45% *cn<sup>+</sup> vg*, 5% *cn<sup>+</sup> vg<sup>+</sup>*, 5% *cn vg*. (b) 45% cinnabar eyes, normal wings; 45% reddish-brown eyes, vestigial wings; 5% reddish-brown eyes, normal wings; 5% cinnabar eyes, vestigial wings.

7.18 In *Drosophila*, the genes *st* (scarlet eyes), *ss* (spineless bristles), and *e* (ebony body) are located on chromosome 3, with map positions as indicated:

<i>st</i>	<i>ss</i>	<i>e</i>
44	58	70

Each of these mutations is recessive to its wild-type allele (*st<sup>+</sup>*, dark red eyes; *ss<sup>+</sup>*, smooth bristles; *e<sup>+</sup>*, gray body). Phenotypically wild-type females with the genotype *st ss e<sup>+</sup>/st<sup>+</sup> st<sup>+</sup> ss<sup>+</sup> e* were crossed with triply recessive males. Predict the phenotypes of the progeny and the frequencies with which they will occur assuming (a) no interference and (b) complete interference.

ANS: In the following enumeration, classes 1 and 2 are parental types, classes 3 and 4 result from a single crossover between *st* and *ss*, classes 5 and 6 result from a single crossover between *ss* and *e*, and classes 7 and 8 result from a double crossover, with one of the exchanges between *st* and *ss* and the other between *ss* and *e*.

Class	Phenotypes	(a) Frequency with No Interference	(b) Frequency with Complete Interference
1	Scarlet, spineless	0.3784	0.37
2	Ebony	0.3784	0.37
3	Scarlet, ebony	0.0616	0.07
4	Spineless	0.0616	0.07

5	Scarlet, spineless, ebony	0.0516	0.06
6	Wild-type	0.0516	0.06
7	Scarlet	0.0084	0
8	Spineless, ebony	0.0084	0

7.19 In maize, the genes *Pl* for purple leaves (dominant over *pl* for green leaves), *sm* for salmon silk (recessive to *Sm* for yellow silk), and *py* for pigmy plant (recessive to *Py* for normal-size plant) are on chromosome 6, with map positions as shown:

<i>pl</i>	<i>sm</i>	<i>py</i>
45	55	65

Hybrids from the cross *Pl sm py/Pl sm py* × *pl Sm Py/pl Sm Py* were testcrossed with *pl sm py/pl sm py* plants. Predict the phenotypes of the offspring and their frequencies assuming (a) no interference and (b) complete interference.

ANS: In the enumeration below, classes 1 and 2 are parental types, classes 3 and 4 result from a single crossover between *Pl* and *Sm*, classes 5 and 6 result from a single crossover between *Sm* and *Py*, and classes 7 and 8 result from a double crossover, with one of the exchanges between *Pl* and *Sm* and the other between *Sm* and *Py*.

Class	Phenotypes	(a) Frequency with No Interference	(b) Frequency with Complete Interference
1	Purple, salmon, pigmy	0.405	0.40
2	Green, yellow, normal	0.405	0.40
3	Purple, yellow, normal	0.045	0.05
4	Green, salmon, pigmy	0.045	0.05
5	Purple, salmon, normal	0.045	0.05
6	Green, yellow, pigmy	0.045	0.05
7	Purple, yellow, pigmy	0.005	0
8	Green, salmon, normal	0.005	0

7.20 In maize, the genes *Tu*, *j2*, and *gl3* are located on chromosome 4 at map positions 101, 106, and 112, respectively. If plants homozygous for the recessive alleles of these genes are crossed with plants homozygous for the dominant alleles, and the F<sub>1</sub> plants are testcrossed to triply recessive plants, what genotypes would you expect,



and in what proportions? Assume that interference is complete over this map interval.

**ANS:** In the following enumeration, classes 1 and 2 are parental types, classes 3 and 4 result from crossing over between *Tu* and *j2*, and classes 5 and 6 result from crossing over between *j2* and *Gl3*; only the chromosome from the triply heterozygous  $F_1$  plant is shown. Because interference is complete, there are no double crossover progeny.

Class	Genotype	Frequency
1	<i>tu j2 gl3</i>	0.445
2	<i>Tu j2 Gl3</i>	0.445
3	<i>tu j2 Gl3</i>	0.025
4	<i>Tu j2 gl3</i>	0.025
5	<i>tu j2 Gl3</i>	0.030
6	<i>Tu j2 gl3</i>	0.030

**7.21** A *Drosophila* geneticist made a cross between females homozygous for three X-linked recessive mutations (*y*, yellow body; *ec*, *echinus* eye shape; *w*, white eye color) and wild-type males. He then mated the  $F_1$  females to triply mutant males and obtained the following results:

Females	Males	Number
+ + + / <i>y ec w</i>	+ + +	475
<i>y ec w</i> / <i>y ec w</i>	<i>y ec w</i>	469
<i>y</i> + + / <i>y ec w</i>	<i>y</i> + +	8
+ <i>ec w</i> / <i>y ec w</i>	+ <i>ec w</i>	7
<i>y</i> + <i>w</i> / <i>y ec w</i>	<i>y</i> + <i>w</i>	18
+ <i>ec</i> + / <i>y ec w</i>	+ <i>ec</i> +	23
+ + <i>w</i> / <i>y ec w</i>	+ + <i>w</i>	0
<i>y ec</i> + / <i>y ec w</i>	<i>y ec</i> +	0

Determine the order of the three loci *y*, *ec*, and *w*, and estimate the distances between them on the linkage map of the X chromosome.

**ANS:** The double crossover classes, which are the two that were not observed, establish that the gene order is *y-w-ec*. Thus, the  $F_1$  females had the genotype *y w ec* / + +. The distance between *y* and *w* is estimated by the frequency of recombination between these two genes:  $(8 + 7)/1000 = 0.015$ ; similarly, the distance between *w* and *ec* is  $(18 + 23)/1000 = 0.041$ . Thus, the genetic map for this segment of the X chromosome is *y*—1.5 cM—*w*—4.1 cM—*ec*.

**7.22** A *Drosophila* geneticist crossed females homozygous for three X-linked mutations (*y*, yellow body; *B*, bar eye shape; *v*, vermilion eye color) to wild-type males. The  $F_1$  females, which had gray bodies and bar eyes with dark red pigment, were then crossed to *y B<sup>+</sup> v* males, yielding the following results:

Phenotype	Number
Yellow, bar, vermilion } wild-type }	546
Yellow } bar, vermilion }	244
Yellow, vermilion } bar }	160
Yellow, bar } vermilion }	50

Determine the order of these three loci on the X chromosome and estimate the distances between them.

**ANS:** The last two classes, consisting of yellow, bar flies and vermilion flies, with a total of 50 progeny, result from double crossovers. Thus, the order of the genes is *y-v-B*, and the  $F_1$  females had the genotype *y v B* / + +. The distance between *y* and *v* is the average number of crossovers between them:  $(244 + 50)/1000 = 29.4$  cM; likewise, the distance between *v* and *B* is  $(160 + 50)/1000 = 21.0$  cM. Thus, the genetic map is *y*—29.4 cM—*v*—21.0 cM—*B*.

**7.23** Female *Drosophila* heterozygous for three recessive mutations *e* (*ebony* body), *st* (*scarlet* eyes), and *ss* (*spineless* bristles) were testcrossed, and the following progeny were obtained:

Phenotype	Number
Wild-type	67
Ebony	8
Ebony, scarlet	68
Ebony, spineless	347
Ebony, scarlet, spineless	78
Scarlet	368
Scarlet, spineless	10
Spineless	54

- What indicates that the genes are linked?
- What was the genotype of the original heterozygous females?
- What is the order of the genes?
- What is the map distance between *e* and *st*?
- What is the map distance between *e* and *ss*?
- What is the coefficient of coincidence?
- Diagram the crosses in this experiment.

**ANS:** (a) Two of the classes (the parental types) vastly outnumber the other six classes (recombinant types); (b) *st* + + / + + *ss e*; (c) *st-ss-e*; (d)  $[(145 + 122) \times 1 + (18) \times 2]/1000 = 30.3$  cM; (e)  $(122 + 18)/1000 = 14.0$  cM; (f)  $(0.018)/(0.163 \times 0.140) = 0.789$ . (g) *st* + + / + + *ss e* females  $\times$  *st ss e/st ss e* males  $\rightarrow$  two parental classes and six recombinant classes.

- 7.24 Consider a female *Drosophila* with the following X chromosome genotype:

$$\frac{w \quad dor^+}{w^+ \quad dor}$$

The recessive alleles *w* and *dor* cause mutant eye colors (white and deep orange, respectively). However, *w* is epistatic over *dor*; that is, the genotypes *w dor* / *Y* and *w dor* / *w dor* have white eyes. If there is 40 percent recombination between *w* and *dor*, what proportion of the sons from this heterozygous female will show a mutant phenotype? What proportion will have either red or deep orange eyes?

**ANS:** The female will produce four kinds of gametes: 30% *w* +, 30% + *dor*, 20% *w dor*, and 20% + +; thus, 80% of the progeny will be mutant (either white or deep orange) and 50% will be pigmented (either red or deep orange).

- 7.25 In *Drosophila*, the X-linked recessive mutations *prune* (*pn*) and *garnet* (*g*) recombine with a frequency of 0.4. Both of these mutations cause the eyes to be brown instead of dark red. Females homozygous for the *pn* mutation were crossed to males hemizygous for the *g* mutation, and the F<sub>1</sub> daughters, all with dark red eyes, were crossed with their brown-eyed brothers. Predict the frequency of sons from this last cross that will have dark red eyes.

**ANS:** The F<sub>1</sub> females are genotypically *pn* + / + *g*. Among their sons, 40 percent will be recombinant for the two X-linked genes, and half of the recombinants will have the wild-type alleles of these genes. Thus, the frequency of sons with dark red eyes will be  $1/2 \times 40\% = 20\%$ .

- 7.26 Assume that in *Drosophila* there are three genes *x*, *y*, and *z*, with each mutant allele recessive to the wild-type allele. A cross between females heterozygous for these three loci and wild-type males yielded the following progeny:

Females	+ + +	1010
Males	+ + +	39
	+ + <i>z</i>	430
	+ <i>y z</i>	32
	<i>x</i> + +	27
	<i>x y</i> +	441
	<i>x y z</i>	31
	Total:	2010

Using these data, construct a linkage map of the three genes and calculate the coefficient of coincidence.

**ANS:** Ignore the female progeny and base the map on the male progeny. The parental types are + + *z* and *x y* +. The two missing classes (+ *y* + and *x* + *z*) must represent double crossovers; thus, the gene order is *y*—*x*—*z*. The distance between *y* and *x* is  $(32 + 27)/1000 = 5.9$  cM and

that between *x* and *z* is  $(31 + 39)/1000 = 7.0$  cM. Thus, the map is *y*—5.9 cM—*x*—7.0 cM—*z*. The coefficient of coincidence is zero.

- 7.27 In the nematode *Caenorhabditis elegans*, the linked genes *dpy* (*dumpy* body) and *unc* (*uncoordinated* behavior) recombine with a frequency *P*. If a repulsion heterozygote carrying recessive mutations in these genes is self-fertilized, what fraction of the offspring will be both dumpy and uncoordinated?

**ANS:**  $(P/2)^2$

- 7.28 In the following testcross, genes *a* and *b* are 20 cM apart, and genes *b* and *c* are 10 cM apart: *a* + *c* / + *b* + × *a b c* / *a b c*. If the coefficient of coincidence is 0.5 over this interval on the linkage map, how many triply homozygous recessive individuals are expected among 1000 progeny?

**ANS:** 5.

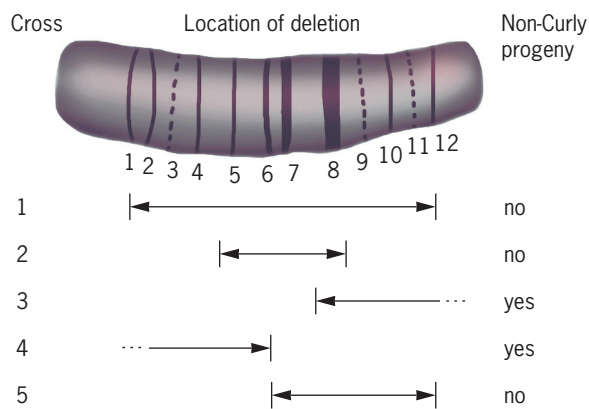
- 7.29 *Drosophila* females heterozygous for three recessive mutations, *a*, *b*, and *c*, were crossed to males homozygous for all three mutations. The cross yielded the following results:

Phenotype	Number
+ + +	75
+ + <i>c</i>	348
+ <i>b c</i>	96
<i>a</i> + +	110
<i>a b</i> +	306
<i>a b c</i>	65

Construct a linkage map showing the correct order of these genes and estimate the distances between them.

**ANS:** From the parental classes, + + *c* and *a b* +, the heterozygous females must have had the genotype + + *c* / *a b* +. The missing classes, + *b* + and *a* + *c*, which would represent double crossovers, establish that the gene order is *b*—*a*—*c*. The distance between *b* and *a* is  $(96 + 110)/1000 = 20.6$  cM and that between *a* and *c* is  $(65 + 75)/1000 = 14.0$  cM. Thus, the genetic map is *b*—20.6 cM—*a*—14.0 cM—*c*.

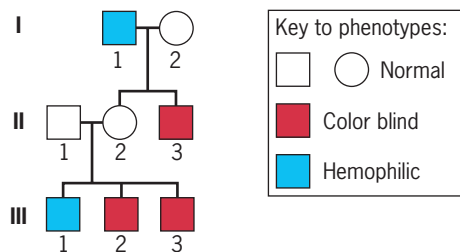
- 7.30 A *Drosophila* second chromosome that carried a recessive lethal mutation, *l(2)g14*, was maintained in a stock with a balancer chromosome marked with a dominant mutation for curly wings. This latter mutation, denoted *Cy*, is also associated with a recessive lethal effect—but this effect is different from that of *l(2)g14*. Thus, *l(2)g14/Cy* flies survive, and they have curly wings. Flies without the *Cy* mutation have straight wings. A researcher crossed *l(2)g14/Cy* females to males that carried second chromosomes with different deletions (all homozygous lethal) balanced over the *Cy* chromosome (genotype *Df/Cy*). Each cross was scored for the presence or absence of progeny with straight wings.



In which band is the lethal mutation  $l(2)g14$  located?

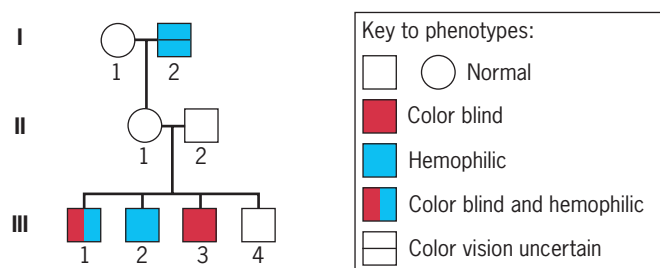
**ANS:** The lethal mutation resides in band 7.

- 7.31** The following pedigree, described in 1937 by C. L. Birch, shows the inheritance of X-linked color blindness and hemophilia in a family. What is the genotype of II-2? Do any of her children provide evidence for recombination between the genes for color blindness and hemophilia?



**ANS:** II-1 has the genotype  $C b/c H$ , that is, she is a repulsion heterozygote for the alleles for color blindness ( $c$ ) and hemophilia ( $b$ ). None of her children are recombinant for these alleles.

- 7.32** The following pedigree, described in 1938 by B. Rath, shows the inheritance of X-linked color blindness and hemophilia in a family. What are the possible genotypes of II-1? For each possible genotype, evaluate the children of II-1 for evidence of recombination between the color blindness and hemophilia genes.



**ANS:** II-1 is either (a)  $C b/c H$  or (b)  $c b/C H$ . Her four sons have the genotypes  $c b$  (1),  $C b$  (2),  $c H$  (3), and  $C H$  (4). If II-1 has the genotype  $C b/c H$ , sons 1 and 4 are recombinant and sons 2 and 3 are nonrecombinant. If II-1 has the genotype  $c b/C H$ , sons 2 and 3 are recombinant and

sons 1 and 4 are nonrecombinant. Either way, the frequency of recombination is 0.5.

- 7.33** A normal woman with a color-blind father married a normal man, and their first child, a boy, had hemophilia. Both color blindness and hemophilia are due to X-linked recessive mutations, and the relevant genes are separated by 10 cM. This couple plans to have a second child. What is the probability that it will have hemophilia? Color blindness? Both hemophilia and color blindness? Neither hemophilia nor color blindness?

**ANS:** The woman is a repulsion heterozygote for the alleles for color blindness and hemophilia—that is, she is  $C b/c H$ . If the woman has a boy, the chance that he will have hemophilia is 0.5 and the chance that he will have color blindness is 0.5. If we specify that the boy have only one of these two conditions, then the chance that he will have color blindness is 0.45. The reason is that the boy will inherit a nonrecombinant X chromosome with a probability of 0.9, and half the nonrecombinant X chromosomes will carry the mutant allele for color blindness and the other half will carry the mutant allele for hemophilia. The chance that the boy will have both conditions is 0.05, and the chance that he will have neither condition is 0.05. The reason is that the boy will inherit a recombinant X chromosome with a probability of 0.1, and half the recombinant X chromosomes will carry both mutant alleles and the other half will carry neither mutant allele.

- 7.34** Two strains of maize, M1 and M2, are homozygous for four recessive mutations,  $a$ ,  $b$ ,  $c$ , and  $d$ , on one of the large chromosomes in the genome. Strain W1 is homozygous for the dominant alleles of these mutations. Hybrids produced by crossing M1 and W1 yield many different classes of recombinants, whereas hybrids produced by crossing M2 and W1 do not yield any recombinants at all. What is the difference between M1 and M2?

**ANS:** M2 carries an inversion that suppresses recombination in the chromosome.

- 7.35** A *Drosophila* geneticist has identified a strain of flies with a large inversion in the left arm of chromosome 3. This inversion includes two mutations,  $e$  (*ebony* body) and  $cd$  (*cardinal* eyes), and is flanked by two other mutations,  $sr$  (*stripe* thorax) on the right and  $ro$  (*rough* eyes) on the left. The geneticist wishes to replace the  $e$  and  $cd$  mutations inside the inversion with their wild-type alleles; he plans to accomplish this by recombining the multiply mutant, inverted chromosome with a wild-type, inversion-free chromosome. What event is the geneticist counting on to achieve his objective? Explain.

**ANS:** A two-strand double crossover within the inversion; the exchange points of the double crossover must lie between the genetic markers and the inversion breakpoints.

## CHAPTER 8

8.1 By what criteria are viruses living? nonliving?

**ANS:** Viruses reproduce and transmit their genes to progeny viruses. They utilize energy provided by host cells and respond to environmental and cellular signals like other living organisms. However, viruses are obligate parasites; they can reproduce only in appropriate host cells.

8.2 How do bacteriophages differ from other viruses?

**ANS:** Bacteriophages reproduce in bacteria; other viruses reproduce in protists, plants, and animals.

8.3 In what ways do the life cycles of bacteriophages T4 and  $\lambda$  differ? In what aspects are they the same?

**ANS:** Bacteriophage T4 is a virulent phage. When it infects a host cell, it reproduces and kills the host cell in the process. Bacteriophage lambda can reproduce and kill the host bacterium—the lytic response—just like phage T4, or it can insert its chromosome into the chromosome of the host and remain there in a dormant state—the lysogenic response.

8.4 How does the structure of the  $\lambda$  prophage differ from the structure of the  $\lambda$  chromosome packaged in the  $\lambda$  head?

**ANS:** The mature (packaged) lambda chromosome and the lambda prophage are circular permutations of one another (see Figure 8.5).

8.5 In what way does the integration of the  $\lambda$  chromosome into the host chromosome during a lysogenic infection differ from crossing over between homologous chromosomes?

**ANS:** The insertion of the phage  $\lambda$  chromosome into the host chromosome is a site-specific recombination process catalyzed by an enzyme that recognizes specific sequences in the  $\lambda$  and *E. coli* chromosomes. Crossing over between homologous chromosomes is not sequence specific. It can occur at many sites along the two chromosomes.

8.6 Geneticists have used mutations that cause altered phenotypes such as white eyes in *Drosophila*, white flowers and wrinkled seeds in peas, and altered coat color in rabbits to determine the locations of genes on the chromosomes of these eukaryotes. What kinds of mutant phenotypes have been used map genes in bacteria?

**ANS:** Three main types of bacterial mutants have been used to map genes in bacteria; these include mutants unable to utilize specific sugars as energy sources (such as lactose), mutants unable to synthesize essential metabolites (these are called auxotrophs), and mutants resistant to drugs and antibiotics. Wild-type bacteria can use almost any sugar as an energy source, can grow on minimal media, and are killed by antibiotics, whereas mutants in genes controlling these processes result in different growth characteristics. These growth phenotypes can be used to map genes in bacteria.

8.7 You have identified three mutations—*a*, *b*, and *c*—in *Streptococcus pneumoniae*. All three are recessive to their wild-type alleles  $a^+$ ,  $b^+$ , and  $c^+$ . You prepare DNA from a wild-type donor strain and use it to transform a strain with genotype *a b c*. You observe  $a^+b^+$  transformants and  $a^+c^+$  transformants, but no  $b^+c^+$  transformants. Are these mutations closely linked? If so, what is their order on the *Streptococcus* chromosome?

**ANS:** The *a*, *b*, and *c* mutations are closely linked and in the order  $b—a—c$  on the chromosome.

8.8 A nutritionally defective *E. coli* strain grows only on a medium containing thymine, whereas another nutritionally defective strain grows only on a medium containing leucine. When these two strains were grown together, a few progeny were able to grow on a minimal medium with neither thymine nor leucine. How can this result be explained?

**ANS:** There are two possible explanations. One possibility is that a spontaneous mutation caused reversion of either auxotrophic strain to the prototrophic condition. Because this requires only one mutation in one cell, this is a possibility, although rare. Another, more likely, possibility is that conjugation occurred between the *E. coli* parental auxotrophic strains. During conjugation, genes from the parental strains recombined. Because each parent had a wild-type gene copy for either thymine or leucine, recombinant progeny containing the wild-type copy of each gene would be able to synthesize both nutrients and grow on minimal medium.

8.9 Assume that you have just demonstrated genetic recombination (e.g., when a strain of genotype *a b*<sup>+</sup> is present with a strain of genotype  $a^+ b$ , some recombinant genotypes,  $a^+ b^+$  and *a b*, are formed) in a previously unstudied species of bacteria. How would you determine whether the observed recombination resulted from transformation, conjugation, or transduction?

**ANS:** Perform two experiments: (1) determine whether the process is sensitive to DNase and (2) determine whether cell contact is required for the process to take place. The cell contact requirement can be tested by a U-tube experiment (see Figure 8.9). If the process is sensitive to DNase, it is similar to transformation. If cell contact is required, it is similar to conjugation. If it is neither sensitive to DNase nor requires cell contact, it is similar to transduction.

8.10 (a) What are the genotypic differences between F<sup>-</sup> cells, F<sup>+</sup> cells, and Hfr cells? (b) What are the phenotypic differences? (c) By what mechanism are F<sup>-</sup> cells converted into F<sup>+</sup> cells? F<sup>+</sup> cells to Hfr cells? Hfr cells to F<sup>+</sup> cells?

**ANS:** (a) F<sup>-</sup> cells, no F factor present; F<sup>+</sup> cells, autonomous F factor; Hfr cells, integrated F factor (see Figure 8.14). (b) F<sup>+</sup> and Hfr cells have F pili; F<sup>-</sup> cells do not. (c) F<sup>-</sup> cells are converted into F<sup>+</sup> cells by the conjugative transfer of F factors from F<sup>+</sup> cells. Hfr cells are formed when

F factors in  $F^+$  cells become integrated into the chromosomes of these cells. Hfr cells become  $F^+$  cells when the integrated F factors exit the chromosome and become autonomous (self-replicating) genetic elements.

- 8.11** (a) Of what use are  $F'$  factors in genetic analysis? (b) How are  $F'$  factors formed? (c) By what mechanism does sex-duction occur?

**ANS:** (a)  $F'$  factors are useful for genetic analyses where two copies of a gene must be present in the same cell, for example, in determining dominance relationships. (b)  $F'$  factors are formed by abnormal excision of F factors from Hfr chromosomes (see Figure 8.21). (c) By the conjugative transfer of an  $F'$  factor from a donor cell to a recipient ( $F^-$ ) cell.

- 8.12** What are the basic differences between generalized transduction and specialized transduction?

**ANS:** Generalized transduction: (1) transducing particles often contain only host DNA; (2) transducing particles may carry any segment of the host chromosome. Thus, all host genes are transduced. Specialized transduction: (1) transducing particles carry a recombinant chromosome, which contains both phage DNA and host DNA; (2) only host genes that are adjacent to the prophage integration site are transduced.

- 8.13** What roles do IS elements play in the integration of F factors?

**ANS:** IS elements (or insertion sequences) are short (800–1400 nucleotide pairs) DNA sequences that are transposable—that is, capable of moving from one position in a chromosome to another position or from one chromosome to another chromosome. IS elements mediate recombination between nonhomologous DNA molecules—for example, between F factors and bacterial chromosomes.

- 8.14** How can bacterial genes be mapped by interrupted mating experiments?

**ANS:** By interrupting conjugation at various times after the donor and recipient cells are mixed (using a blender or other form of agitation), one can determine the length of time required to transfer a given genetic marker from an Hfr cell to an  $F^-$ .

- 8.15** What does the term *cotransduction* mean? How can cotransduction frequencies be used to map genetic markers?

**ANS:** Cotransduction refers to the simultaneous transduction of two different genetic markers to a single recipient cell. Since bacteriophage particles can package only 1/100 to 1/50 of the total bacterial chromosome, only markers that are relatively closely linked can be cotransduced. The frequency of cotransduction of any two markers will be an inverse function of the distance between them on the chromosome. As such, this

frequency can be used as an estimate of the linkage distance. Specific cotransduction-linkage functions must be prepared for each phage–host system studied.

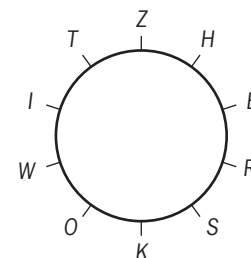
- 8.16** In *E. coli*, the ability to utilize lactose as a carbon source requires the presence of the enzymes  $\beta$ -galactosidase and  $\beta$ -galactoside permease. These enzymes are encoded by two closely linked genes, *lacZ* and *lacY*, respectively. Another gene, *proC*, controls, in part, the ability of *E. coli* cells to synthesize the amino acid proline. The alleles *str<sup>r</sup>* and *str<sup>s</sup>*, respectively, control resistance and sensitivity to streptomycin. Hfr H is known to transfer the two *lac* genes, *proC*, and *str*, in that order, during conjugation. A cross was made between Hfr H of genotype *lacZ<sup>-</sup> lacY<sup>+</sup> proC<sup>+</sup> str<sup>s</sup>* and an  $F^-$  strain of genotype *lacZ<sup>+</sup> lacY<sup>-</sup> proC<sup>-</sup> str<sup>r</sup>*. After about 2 hours, the mixture was diluted and plated out on medium containing streptomycin but no proline. When the resulting *proC<sup>+</sup> str<sup>r</sup>* recombinant colonies were checked for their ability to grow on medium containing lactose as the sole carbon source, very few of them were capable of fermenting lactose. When the reciprocal cross (Hfr H *lacZ<sup>+</sup> lacY<sup>-</sup> proC<sup>+</sup> str<sup>s</sup>* X  $F^-$  *lacZ<sup>-</sup> lacY<sup>+</sup> proC<sup>-</sup> str<sup>r</sup>*) was done, many of the *proC<sup>+</sup> str<sup>r</sup>* recombinants were able to grow on medium containing lactose as the sole carbon source. What is the order of the *lacZ* and *lacY* genes relative to *proC*?

**ANS:** *lacY*—*lacZ*—*proC*.

- 8.17** An  $F^+$  strain, marked at 10 loci, gives rise spontaneously to Hfr progeny whenever the F factor becomes incorporated into the chromosome of the  $F^+$  strain. The F factor can integrate into the circular chromosome at many points, so that the resulting Hfr strains transfer the genetic markers in different orders. For any Hfr strain, the order of markers entering a recipient cell can be determined by interrupted mating experiments. From the following data for several Hfr strains derived from the same  $F^+$ , determine the order of markers in the  $F^+$  strain.

Hfr Strain	Markers Donated in order
1	—Z—H—E—R—
2	—O—K—S—R—
3	—K—O—W—I—
4	—Z—T—I—W—
5	—H—Z—T—I—

**ANS:**



8.18 The data in the following table were obtained from three-point transduction tests made to determine the order of mutant sites in the *A* gene encoding the  $\alpha$  subunit of tryptophan synthetase in *E. coli*. *Anth* is a linked, unselected marker. In each cross, *trp*<sup>+</sup> recombinants were selected and then scored for the *anth* marker (*anth*<sup>+</sup> or *anth*<sup>-</sup>). What is the linear order of *anth* and the three mutant alleles of the *A* gene indicated by the data in the table?

Cross	Donor Markers	Recipient Markers	<i>anth</i> Allele in <i>trp</i> <sup>+</sup> Recombinants	% <i>anth</i> <sup>+</sup>
1	<i>anth</i> <sup>+</sup> — A34	<i>anth</i> <sup>-</sup> — A223	72 <i>anth</i> <sup>+</sup> : 332 <i>anth</i> <sup>-</sup>	18
2	<i>anth</i> <sup>+</sup> — A46	<i>anth</i> <sup>-</sup> — A223	196 <i>anth</i> <sup>+</sup> : 180 <i>anth</i> <sup>-</sup>	52
3	<i>anth</i> <sup>+</sup> — A223	<i>anth</i> <sup>-</sup> — A34	380 <i>anth</i> <sup>+</sup> : 379 <i>anth</i> <sup>-</sup>	50
4	<i>anth</i> <sup>+</sup> — A223	<i>anth</i> <sup>-</sup> — A46	60 <i>anth</i> <sup>+</sup> : 280 <i>anth</i> <sup>-</sup>	20

ANS: *anth*—A34—A223—A46.

8.19 Bacteriophage P1 mediates generalized transduction in *E. coli*. A P1 transducing lysate was prepared by growing P1 phage on *pur*<sup>+</sup> *pro*<sup>-</sup> *bis*<sup>-</sup> bacteria. Genes *pur*, *pro*, and *bis* encode enzymes required for the synthesis of purines, proline, and histidine, respectively. The phage and transducing particles in this lysate were then allowed to infect *pur*<sup>-</sup> *pro*<sup>+</sup> *bis*<sup>+</sup> cells. After incubating the infected bacteria for a period of time sufficient to allow transduction to occur, they were plated on minimal medium supplemented with proline and histidine, but no purines to select for *pur*<sup>+</sup> transductants. The *pur*<sup>+</sup> colonies were then transferred to minimal medium with and without proline and with and without histidine to determine the frequencies of each of the outside markers. Given the following results, what is the order of the three genes on the *E. coli* chromosome?

Genotype	Number Observed
<i>pro</i> <sup>+</sup> <i>bis</i> <sup>+</sup>	100
<i>pro</i> <sup>+</sup> <i>bis</i> <sup>+</sup>	22
<i>pro</i> <sup>+</sup> <i>bis</i> <sup>-</sup>	150
<i>pro</i> <sup>-</sup> <i>bis</i> <sup>-</sup>	1

ANS: *pro*—*pur*—*bis*.

8.20 Two additional mutations in the *trp A* gene of *E. coli*, *trp A58* and *trp A487*, were ordered relative to *trp A223* and the outside marker *anth* by three-factor transduction

crosses as described in Problem 8.18. The results of these crosses are summarized in the following table. What is the linear order of *anth* and the three mutant sites in the *trp A* gene?

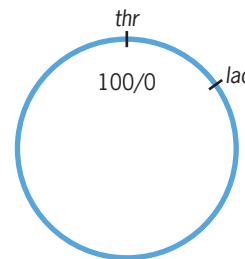
Cross	Donor Markers	Recipient Markers	<i>anth</i> Allele in <i>trp</i> <sup>+</sup> Recombinants	% <i>anth</i> <sup>-</sup>
1	<i>anth</i> <sup>+</sup> — A487	<i>anth</i> <sup>-</sup> — A223	72 <i>anth</i> <sup>+</sup> : 332 <i>anth</i> <sup>-</sup>	82
2	<i>anth</i> <sup>+</sup> — A58	<i>anth</i> <sup>-</sup> — A223	196 <i>anth</i> <sup>+</sup> : 180 <i>anth</i> <sup>-</sup>	48
3	<i>anth</i> <sup>+</sup> — A223	<i>anth</i> <sup>-</sup> — A487	380 <i>anth</i> <sup>+</sup> : 379 <i>anth</i> <sup>-</sup>	50
4	<i>anth</i> <sup>+</sup> — A223	<i>anth</i> <sup>-</sup> — A58	60 <i>anth</i> <sup>+</sup> : 280 <i>anth</i> <sup>-</sup>	80

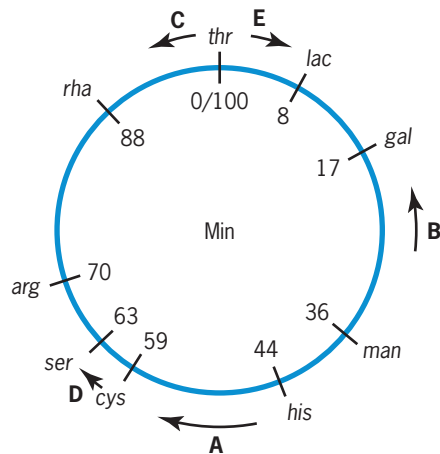
ANS: *anth*—A487—A223—A58.

8.21 You have identified a mutant *E. coli* strain that cannot synthesize histidine (His<sup>-</sup>). To determine the location of the *bis*<sup>-</sup> mutation on the *E. coli* chromosome, you perform interrupted mating experiments with five different Hfr strains. The following chart shows the time of entry (minutes, in parentheses) of the wild-type alleles of the first five markers (mutant genes) into the His<sup>-</sup> strain.

Hfr A	-----	<i>bis</i> (1)	<i>man</i> (9)	<i>gal</i> (28)	<i>lac</i> (37)	<i>tbr</i> (45)
Hfr B	-----	<i>man</i> (15)	<i>bis</i> (23)	<i>cys</i> (38)	<i>ser</i> (42)	<i>arg</i> (49)
Hfr C	-----	<i>tbr</i> (3)	<i>lac</i> (11)	<i>gal</i> (20)	<i>man</i> (39)	<i>bis</i> (47)
Hfr D	-----	<i>cys</i> (3)	<i>bis</i> (18)	<i>man</i> (26)	<i>gal</i> (45)	<i>lac</i> (54)
Hfr E	-----	<i>tbr</i> (6)	<i>rha</i> (18)	<i>arg</i> (36)	<i>ser</i> (43)	<i>cys</i> (47)

On the map below of the circular *E. coli* chromosome, indicate (1) the relative location of each gene relative to *tbr* (located at 0/100 Min), (2) the position where the sex factor is integrated in each of the five Hfr's, and (3) the direction of chromosome transfer for each Hfr (indicate direction with an arrow or arrowhead).



**ANS:**


- 8.22 Mutations *nrd 11* (gene *nrd B*, encoding the beta subunit of the enzyme ribonucleotide reductase), *am M69* (gene 63, encoding a protein that aids tail-fiber attachment), and *nd 28* (gene *denA*, encoding the enzyme endonuclease II) are known to be located between gene 31 and gene 32 on the bacteriophage T4 chromosome. Mutations *am N54* and *am A453* are located in genes 31 and 32, respectively. Given the three-factor cross data in the following table, what is the linear order of the five mutant sites?

#### Three-Factor Cross Data

Cross	% Recombination <sup>a</sup>
1. <i>am A453—am M69</i> × <i>nrd 11</i>	2.6
2. <i>am A453—am M69</i> × <i>nd 28</i>	4.2
3. <i>am A453—am M69</i> × <i>nd 28</i>	2.5
4. <i>am A453—nd 28</i> × <i>am M69</i>	3.5
5. <i>am A453—nrd 11</i> × <i>nd 28</i>	2.9
6. <i>am A453—nd 28</i> × <i>nrd 11</i>	2.1
7. <i>am N54—am M69</i> × <i>nrd 11</i>	3.5
8. <i>am N54—nrd 11</i> × <i>am M69</i>	1.9
9. <i>am N54—nd 28</i> × <i>am M69</i>	1.7
10. <i>am N54—am M69</i> × <i>nd 28</i>	2.7
11. <i>am N54—nd 28</i> × <i>nrd 11</i>	2.9
12. <i>am N54—nrd 11</i> × <i>nd 28</i>	1.9

<sup>a</sup>All recombination frequencies are given as

$$2 \frac{(\text{wild type progeny})}{\text{total progeny}} \times 100.$$

**ANS:** *amA453—nrd11—nd28—amM69—amN54*.

## CHAPTER 9

- 9.1 (a) How did the transformation experiments of Griffith differ from those of Avery and his associates? (b) What was the significant contribution of each? (c) Why was Griffith's work not evidence for DNA as the genetic material, whereas the experiments of Avery and coworkers provided direct proof that DNA carried the genetic information?

**ANS:** (a) Griffith's *in vivo* experiments demonstrated the occurrence of transformation in pneumococcus. They provided no indication as to the molecular basis of the transformation phenomenon. Avery and colleagues carried out *in vitro* experiments, employing biochemical analyses to demonstrate that transformation was mediated by DNA. (b) Griffith showed that a transforming substance existed; Avery *et al.* defined it as DNA. (c) Griffith's experiments did not include any attempt to characterize the substance responsible for transformation. Avery *et al.* isolated DNA in "pure" form and demonstrated that it could mediate transformation.

- 9.2 A cell-free extract is prepared from Type IIIS pneumococcal cells. What effect will treatment of this extract with (a) protease, (b) RNase, and (c) DNase have on its subsequent capacity to transform recipient Type IIR cells to Type IIIS? Why?

**ANS:** (a) No effect; (b) no effect; (c) DNase will destroy the capacity of the extract to transform type IIR cells to Type IIIS by degrading the DNA in the extract. Protease and RNase will degrade the proteins and RNA, respectively, in the extract. They will have no effect, since the proteins and RNA are not involved in transformation.

- 9.3 How could it be demonstrated that the mixing of heat-killed Type III pneumococcus with live Type II resulted in a transfer of genetic material from Type III to Type II rather than a restoration of viability to Type III by Type II?

**ANS:** Purified DNA from Type III cells was shown to be sufficient to transform Type II cells. This occurred in the absence of any dead Type III cells.

- 9.4 What is the macromolecular composition of a bacterial virus or bacteriophage such as phage T2?

**ANS:** About 1/2 protein, 1/2 DNA. A single long molecule of DNA is enclosed within a complex "coat" composed of many proteins.

- 9.5 (a) What was the objective of the experiment carried out by Hershey and Chase? (b) How was the objective accomplished? (c) What is the significance of this experiment?

**ANS:** (a) The objective was to determine whether the genetic material was DNA or protein. (b) By labeling phosphorus, a constituent of DNA, and sulfur, a constituent of protein, in a virus, it was possible to demonstrate that only the labeled phosphorus was introduced into the

host cell during the viral reproductive cycle. The DNA was enough to produce new phages. (c) Therefore DNA, not protein, is the genetic material.

- 9.6** How did the reconstitution experiment of Fraenkel–Conrat and colleagues show that the genetic information of tobacco mosaic virus (TMV) is stored in its RNA rather than its protein?

**ANS:** When tobacco leaves were infected with reconstituted virus particles containing RNA from type A viruses and protein from type B viruses, the progeny viruses were type A, showing that RNA, not protein, carries the genetic information in TMV.

- 9.7** (a) What background material did Watson and Crick have available for developing a model of DNA? (b) What was their contribution to building the model?

**ANS:** (a) The ladder-like pattern was known from X-ray diffraction studies. Chemical analyses had shown that a 1:1 relationship existed between the organic bases adenine and thymine and between cytosine and guanine. Physical data concerning the length of each spiral and the stacking of bases were also available. (b) Watson and Crick developed the model of a double helix, with the rigid strands of sugar and phosphorus forming spirals around an axis, and hydrogen bonds connecting the complementary bases in nucleotide pairs.

- 9.8** (a) Why did Watson and Crick choose a double helix for their model of DNA structure? (b) Why were hydrogen bonds placed in the model to connect the bases?

**ANS:** (a) A multistranded, spiral structure was suggested by the X-ray diffraction patterns. A double-stranded helix with specific base-pairing nicely fits the 1:1 stoichiometry observed for A:T and G:C in DNA. (b) Use of the known hydrogen-bonding potential of the bases provided a means of holding the two complementary strands in a stable configuration in such a double helix.

- 9.9** (a) If a virus particle contained double-stranded DNA with 200,000 base pairs, how many nucleotides would be present? (b) How many complete spirals would occur on each strand? (c) How many atoms of phosphorus would be present? (d) What would be the length of the DNA configuration in the virus?

**ANS:** (a) 400,000; (b) 20,000; (c) 400,000; (d) 68,000 nm.

- 9.10** What are the differences between DNA and RNA?

**ANS:** DNA has one atom less of oxygen than RNA in the sugar part of the molecule; the sugar in DNA is 2-deoxyribose, whereas the sugar in RNA is ribose. In DNA, thymine replaces the uracil that is present in RNA. (In certain bacteriophages, DNA-containing uracil is present.) DNA is most frequently double-stranded, but bacteriophages such as  $\Phi$ X174 contain single-stranded DNA. RNA is most frequently single-stranded. Some viruses, such as the Reoviruses, however, contain double-stranded RNA chromosomes.

- 9.11** RNA was extracted from TMV (tobacco mosaic virus) particles and found to contain 20 percent cytosine (20 percent of the bases were cytosine). With this information, is it possible to predict what percentage of the bases in TMV are adenine? If so, what percentage? If not, why not?

**ANS:** No. TMV RNA is single-stranded. Thus, the base-pair stoichiometry of DNA does not apply.

- 9.12** DNA was extracted from cells of *Staphylococcus afermentans* and analyzed for base composition. It was found that 37 percent of the bases are cytosine. With this information, is it possible to predict what percentage of the bases are adenine? If so, what percentage? If not, why not?

**ANS:** Yes. Because DNA in bacteria is double-stranded, the 1:1 base-pair stoichiometry applies. Therefore, if 37% of the bases are cytosine, then 37% are guanine. This means that the remaining 26% of the bases are adenine and thymine. Thus,  $26\%/2 = 13\%$  of the bases are adenine.

- 9.13** If one strand of DNA in the Watson–Crick double helix has a base sequence of 5-GTCATGAC-3, what is the base sequence of the complementary strand?

**ANS:** 3'-CAGTACTG-5'

- 9.14** Indicate whether each of the following statements about the structure of DNA is true or false. (Each letter is used to refer to the concentration of that base in DNA.)

(a)  $A + T = G + C$

(b)  $A = G; C = T$

(c)  $A/T = C/G$

(d)  $T/A = C/G$

(e)  $A + G = C + T$

(f)  $G/C = 1$

(g)  $A = T$  within each single strand.

(h) Hydrogen bonding provides stability to the double helix in aqueous cytoplasm.

(i) Hydrophobic bonding provides stability to the double helix in aqueous cytoplasm.

(j) When separated, the two strands of a double helix are identical.

(k) Once the base sequence of one strand of a DNA double helix is known, the base sequence of the second strand can be deduced.

(l) The structure of a DNA double helix is invariant. (m) Each nucleotide pair contains two phosphate groups, two deoxyribose molecules, and two bases.

**ANS:** (a) False (b) False (c) True (d) True (e) True  
(f) True (g) False (h) True (i) True (j) False  
(k) True (l) False (m) True

- 9.15** The nucleic acids from various viruses were extracted and examined to determine their base composition. Given the following results, what can you hypothesize about the physical nature of the nucleic acids from these viruses?



(a) 35% A, 35% T, 15% G, and 15% C. (b) 35% A, 15% T, 25% G, and 25% C. (c) 35% A, 30% U, 30% G, and 5% C.

**ANS:** (a) Double-stranded DNA; (b) single-stranded DNA; (c) single-stranded RNA.

**9.16** Compare and contrast the structures of the A, B, and Z forms of DNA.

**ANS:** The B form of DNA helix is that proposed by Watson and Crick and is the conformation that DNA takes under physiological conditions. It is a right-handed double helical coil with 10 bases per turn of the helix and a diameter of 1.9 nm. It has a major and a minor groove. Z-DNA is left-handed, has 12 bases per turn, a single deep groove, and is 1.8 nm in diameter. Its sugar-phosphate backbone takes a zigzagged path, and it is G:C rich. A-DNA is a right-handed helix with 11 base pairs per turn. It is a shorter, thicker double helix with a diameter of 0.23 nm and has a narrow, deep major groove and a broad, shallow minor groove. A-DNA forms *in vitro* under high salt concentrations or in a partially dehydrated state.

**9.17** The temperature at which one-half of a double-stranded DNA molecule has been denatured is called the melting temperature,  $T_m$ . Why does  $T_m$  depend directly on the GC content of the DNA?

**ANS:** The value of  $T_m$  increases with the GC content because GC base pairs, connected by three hydrogen bonds, are stronger than AT base pairs connected by two hydrogen bonds.

**9.18** A diploid rye plant, *Secale cereale*, has  $2n = 14$  chromosomes and approximately  $1.6 \times 10^{10}$  bp of DNA. How much DNA is in a nucleus of a rye cell at (a) mitotic metaphase, (b) meiotic metaphase I, (c) mitotic telophase, and (d) meiotic telophase II?

**ANS:** (a)  $3.2 \times 10^{10}$  bp (b)  $3.2 \times 10^{10}$  bp (c)  $1.6 \times 10^{10}$  bp (d)  $0.8 \times 10^{10}$  bp

**9.19** The available evidence indicates that each eukaryotic chromosome (excluding polytene chromosomes) contains a single giant molecule of DNA. What different levels of organization of this DNA molecule are apparent in chromosomes of eukaryotes at various times during the cell cycle?

**ANS:** DNA during interphase is not yet organized into individual chromosomes but consists of a series of ellipsoidal “beads on a string” that form an 11-nm fiber. Here, 146 bp of DNA is wrapped 1.65 turns around the nucleosome core of eight histones. However, during metaphase of meiosis and mitosis, DNA becomes organized into chromosomes. The 11-nm fiber is folded and supercoiled to produce a 30-nm chromatin fiber, the basic structural unit of the metaphase chromosome. A third and final level of packaging involves nonhistone chromosomal proteins that form a scaffold to condense the 30-nm fibers into tightly packaged metaphase chromosomes, the highest level of DNA condensation observed.

**9.20** A diploid nucleus of *Drosophila melanogaster* contains about  $3.4 \times 10^8$  nucleotide pairs. Assume (1) that all nuclear DNA is packaged in nucleosomes and (2) that an average internucleosome linker size is 60 nucleotide pairs. How many nucleosomes would be present in a diploid nucleus of *D. melanogaster*? How many molecules of histone H2a, H2b, H3, and H4 would be required?

**ANS:** In the diploid nucleus of *D. melanogaster*,  $1.65 \times 10^6$  nucleosomes would be present; these would contain  $3.3 \times 10^6$  molecules of each histone, H2a, H2b, H3, and H4.

**9.21** The relationship between the melting  $T_m$  and GC content can be expressed, in its much simplified form, by the formula  $T_m = 69 + 0.41 (\% \text{ GC})$ . (a) Calculate the melting temperature of *E. coli* DNA that has about 50% GC. (b) Estimate the %GC of DNA from a human kidney cell where  $T_m = 85^\circ\text{C}$ .

**ANS:** (a)  $89.5^\circ\text{C}$ . (b) About 39%

**9.22** Experimental evidence indicates that most highly repetitive DNA sequences in the chromosomes of eukaryotes do not produce any RNA or protein products. What does this indicate about the function of highly repetitive DNA?

**ANS:** It indicates that most highly repetitive DNA sequences do not contain structural genes specifying RNA and polypeptide gene products.

**9.23** The satellite DNAs of *Drosophila virilis* can be isolated, essentially free of main-fraction DNA, by density-gradient centrifugation. If these satellite DNAs are sheared into approximately 40-nucleotide-pair-long fragments and are analyzed in denaturation-renaturation experiments, how would you expect their hybridization kinetics to compare with the renaturation kinetics observed using similarly sheared main-fraction DNA under the same conditions? Why?

**ANS:** The satellite DNA fragments would renature much more rapidly than the main-fraction DNA fragments. In *D. virilis* satellite DNAs, all three have repeating hepta-nucleotide-pair sequences. Thus, essentially every 40 nucleotide-long (average) single-stranded fragment from one strand will have a sequence complementary (in part) with every single-stranded fragment from the complementary strand. Many of the nucleotide-pair sequences in main-fraction DNA will be unique sequences (present only once in the genome).

**9.24** (a) What functions do (1) centromeres and (2) telomeres provide? (b) Do telomeres have any unique structural features? (c) When chromosomes are broken by exposure to high-energy radiation such as X rays, the resulting broken ends exhibit a pronounced tendency to stick to each other and fuse. Why might this occur?

**ANS:** (a) (1) Centromeres function as spindle-fiber attachment sites on chromosomes; they are required for the separation of homologous chromosomes to opposite poles of

the spindle during anaphase I of meiosis and for the separation of sister chromatids during anaphase of mitosis and anaphase II of meiosis. (2) Telomeres provide at least three important functions: (i) prevention of exonucleolytic degradation of the ends of the linear DNA molecules in eukaryotic chromosomes, (ii) prevention of the fusion of ends of DNA molecules of different chromosomes, and (iii) provision of a mechanism for replication of the distal tips of linear DNA molecules in eukaryotic chromosomes. (b) Yes. Most telomeres studied to date contain DNA sequence repeat units (e.g., TTAGGG in human chromosomes), and at least in some species, telomeres terminate with single-stranded 3' overhangs that form "hairpin" structures. The bases in these hairpins exhibit unique patterns of methylation that presumably contribute to the structure and stability of telomeres. (c) The broken ends resulting from irradiation will not contain telomeres; as a result, the free ends of the DNA molecules are apparently subject to the activities of enzymes such as exonucleases, ligases, and so on, which modify the ends. They can regain stability by fusing to broken ends of other DNA molecules that contain terminal telomere sequences.

**9.25** Are eukaryotic chromosomes metabolically most active during prophase, metaphase, anaphase, telophase, or interphase?

**ANS:** Interphase. Chromosomes are for the most part metabolically inactive (exhibiting little transcription) during the various stages of condensation in mitosis and meiosis.

**9.26** Are the scaffolds of eukaryotic chromosomes composed of histone or nonhistone chromosomal proteins? How has this been determined experimentally?

**ANS:** Nonhistone chromosomal proteins. The "scaffold" structures of metaphase chromosomes can be observed by light microscopy after removal of the histones by differential extraction procedures.

**9.27** (a) Which class of chromosomal proteins, histones or nonhistones, is the more highly conserved in different eukaryotic species? Why might this difference be expected? (b) If one compares the histone and nonhistone chromosomal proteins of chromatin isolated from different tissues or cell types of a given eukaryotic organism, which class of proteins will exhibit the greater heterogeneity? Why are both classes of proteins not expected to be equally homogeneous in chromosomes from different tissues or cell types?

**ANS:** (a) Histones have been highly conserved throughout the evolution of eukaryotes. A major function of histones is to package DNA into nucleosomes and chromatin fibers. Since DNA is composed of the same four nucleotides and has the same basic structure in all eukaryotes, one might expect that the proteins that play a structural role

in packaging this DNA would be similarly conserved. (b) The nonhistone chromosomal proteins exhibit the greater heterogeneity in chromatin from different tissues and cell types of an organism. The histone composition is largely the same in all cell types within a given species—consistent with the role of histones in packaging DNA into nucleosomes. The nonhistone chromosomal proteins include proteins that regulate gene expression. Because different sets of genes are transcribed in different cell types, one would expect heterogeneity in some of the nonhistone chromosomal proteins of different tissues.

**9.28** (a) If the haploid human genome contains  $3 \times 10^9$  nucleotide pairs and the average molecular weight of a nucleotide pair is 660, how many copies of the human genome are present, on average, in 1 mg of human DNA? (b) What is the weight of one copy of the human genome? (c) If the haploid genome of the small plant *Arabidopsis thaliana* contains  $7.7 \times 10^7$  nucleotide pairs, how many copies of the *A. thaliana* genome are present, on average, in 1 mg of *A. thaliana* DNA? (d) What is the weight of one copy of the *A. thaliana* genome? (e) Of what importance are calculations of the above type to geneticists?

**ANS:** (a) One microgram of human DNA will contain, on average,  $3.04 \times 10^5$  copies of the genome. Using an average molecular weight per nucleotide pair of 660, the molecular weight of the entire human genome is  $1.98 \times 10^{12}$  ( $3 \times 10^9 \times 660$ ). Thus,  $1.98 \times 10^{12}$  g (1 "mole" = number of grams equivalent to the "molecular" weight) of human DNA will contain, on average,  $6.02 \times 10^{23}$  molecules [Avogadro's number = number of molecules (here, copies of the genome) present in one "mole" of a substance]. One gram will contain on average  $(3.04 \times 10^{11})(6.02 \times 10^{23}/1.98 \times 10^{12})$  copies of the genome; thus, 1  $\mu$ g will contain, on average,  $3.04 \times 10^5$  copies of the human genome. (b) One copy of the human genome weighs approximately ( $3.3 \times 10^{-12}$  g) ( $1.98 \times 10^{12}$  g per "mole"/ $6.02 \times 10^{23}$  molecules per "mole") or  $3.3 \times 10^{-6}$   $\mu$ g. (c) By analogous calculations, 1 g of *A. thaliana* DNA contains, on average,  $1.18 \times 10^7$  copies of the genome. (d) Similarly, one copy of the *A. thaliana* genome weighs approximately  $8.4 \times 10^{-8}$   $\mu$ g. (e) In carrying out molecular analyses of the structures of genomes, geneticists frequently need to know how many copies of a genome are present, on average, in a given quantity of DNA.

## CHAPTER 10

**10.1** DNA polymerase I of *E. coli* is a single polypeptide of molecular weight 103,000.

(a) What enzymatic activities other than polymerase activity does this polypeptide possess? (b) What are the *in vivo* functions of these activities? (c) Are these activities of major importance to an *E. coli* cell? Why?

**ANS:** (a) Both  $3' \rightarrow 5'$  and  $5' \rightarrow 3'$  exonuclease activities. (b) The  $3' \rightarrow 5'$  exonuclease “proofreads” the nascent DNA strand during its synthesis. If a mismatched base pair occurs at the  $3'$ -OH end of the primer, the  $3' \rightarrow 5'$  exonuclease removes the incorrect terminal nucleotide before polymerization proceeds again. The  $5' \rightarrow 3'$  exonuclease is responsible for the removal of RNA primers during DNA replication and functions in pathways involved in the repair of damaged DNA (see Chapter 13). (c) Yes, both exonuclease activities appear to be very important. Without the  $3' \rightarrow 5'$  proofreading activity during replication, an intolerable mutation frequency would occur. The  $5' \rightarrow 3'$  exonuclease activity is essential to the survival of the cell. Conditional mutations that alter the  $5' \rightarrow 3'$  exonuclease activity of DNA polymerase I are lethal to the cell under conditions where the exonuclease is nonfunctional.

**10.2** *Escherichia coli* cells are grown for many generations in a medium in which the only available nitrogen is the heavy isotope  $^{15}\text{N}$ . They are then transferred to a medium containing  $^{14}\text{N}$  as the only source of nitrogen.

(a) What distribution of  $^{15}\text{N}$  and  $^{14}\text{N}$  would be expected in the DNA molecules of cells that had grown for one generation in the  $^{14}\text{N}$ -containing medium assuming that DNA replication was (i) conservative, (ii) semiconservative, or (iii) dispersive?

(b) What distribution would be expected after two generations of growth in the  $^{14}\text{N}$ -containing medium assuming (i) conservative, (ii) semiconservative, or (iii) dispersive replication?

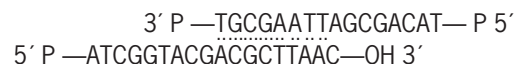
**ANS:** (a) (i) One-half of the DNA molecules with  $^{15}\text{N}$  in both strands and the other half with  $^{14}\text{N}$  in both strands; (ii) all DNA molecules with one strand containing  $^{15}\text{N}$  and the complementary strand containing  $^{14}\text{N}$ ; (iii) all DNA molecules with both strands containing roughly equal amounts of  $^{15}\text{N}$  and  $^{14}\text{N}$ . (b) (i)  $1/4$  of the DNA molecules with  $^{15}\text{N}$  in both strands and  $3/4$  with  $^{14}\text{N}$  in both strands; (ii) half of the DNA molecules with one strand containing  $^{15}\text{N}$  and the complementary strand containing  $^{14}\text{N}$  and the other half with  $^{14}\text{N}$  in both strands; (iii) all DNA molecules with both strands containing about  $1/4$   $^{15}\text{N}$  and  $3/4$   $^{14}\text{N}$ .

**10.3** Why do DNA molecules containing  $^{15}\text{N}$  band at a different position than DNA molecules containing  $^{14}\text{N}$  when centrifuged to equilibrium in  $6M$   $\text{CsCl}$ ?

**ANS:**  $^{15}\text{N}$  contains eight neutrons instead of the seven neutrons in the normal isotope of nitrogen,  $^{14}\text{N}$ . Therefore,  $^{15}\text{N}$  has an atomic mass of about 15, whereas  $^{14}\text{N}$  has a mass of about 14. This difference means that purines and pyrimidines containing  $^{15}\text{N}$  have a greater density (weight per unit volume) than those containing  $^{14}\text{N}$ . Equilibrium density-gradient centrifugation in  $6M$   $\text{CsCl}$  separates DNAs or other macromolecules based on their

densities, and *E. coli* DNA, for example, that contains  $^{15}\text{N}$  has a density of  $1.724 \text{ g/cm}^3$ , whereas *E. coli* DNA that contains  $^{14}\text{N}$  has a density of  $1.710 \text{ g/cm}^3$ .

**10.4** A DNA template plus primer with the structure



(where P = a phosphate group) is placed in an *in vitro* DNA synthesis system ( $\text{Mg}^{2+}$ , an excess of the four deoxyribonucleoside triphosphates, etc.) containing a mutant form of *E. coli* DNA polymerase I that lacks exonuclease activity. The polymerase and exonuclease activities of this aberrant enzyme are identical to those of normal *E. coli* DNA polymerase I. It simply has no exonuclease activity.

(a) What will be the structure of the final product?

(b) What will be the first step in the reaction sequence?

**ANS:** (a) 
$$\begin{array}{l} 3' \text{ (P) — TGCGAATTAGCGACAT — (P) } 5' \\ 5' \text{ (P) — ATCGGTACGACGCTTAATCGCTGTA — OH } 3' \end{array}$$

Note that DNA synthesis will *not* occur on the left end since the  $3'$ -terminus of the potential primer strand is blocked with a phosphate group—all DNA polymerases require a free  $3'$ -OH terminus.

(b) The first step will be the removal of the mismatched C (exiting as dCMP) from the  $3'$ -OH primer terminus by the  $3' \rightarrow 5'$  exonuclease (“proofreading”) activity.

**10.5** How might continuous and discontinuous modes of DNA replication be distinguished experimentally?

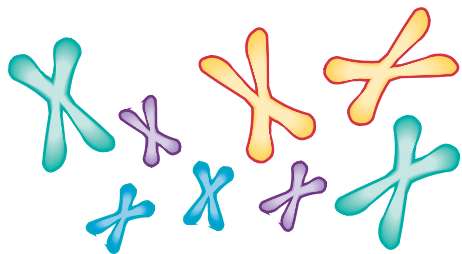
**ANS:** If nascent DNA is labeled by exposure to  $^3\text{H}$ -thymidine for very short periods of time, continuous replication predicts that the label would be incorporated into chromosome-sized DNA molecules, whereas discontinuous replication predicts that the label would first appear in small pieces of nascent DNA (prior to covalent joining, catalyzed by polynucleotide ligase).

**10.6** *E. coli* cells contain five different DNA polymerases—I, II, III, IV, and V. Which of these enzymes catalyzes the semiconservative replication of the bacterial chromosome during cell division? What are the functions of the other four DNA polymerases in *E. coli*?

**ANS:** DNA polymerase III is the true replicase. DNA polymerase I removes the RNA primers and replaces them with DNA. The other DNA polymerases play important roles in DNA repair pathways (see Chapter 13).

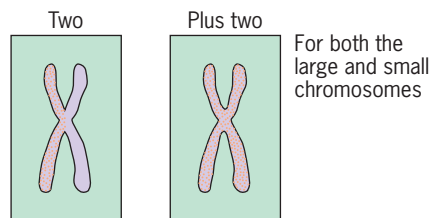
**10.7** The Boston barberry is an imaginary plant with a diploid chromosome number of 4, and Boston barberry cells are easily grown in suspended cell cultures.  $^3\text{H}$ -thymidine was added to the culture medium in which a G1-stage cell of this plant was growing. After one cell

generation of growth in  $^3\text{H}$ -thymidine-containing medium, colchicine was added to the culture medium. The medium now contained both  $^3\text{H}$ -thymidine and colchicine. After two “generations” of growth in  $^3\text{H}$ -thymidine-containing medium (the second “generation” occurring in the presence of colchicine as well), the two progeny cells (each now containing eight chromosomes) were transferred to culture medium containing nonradioactive thymidine ( $^3\text{H}$ -thymidine) plus colchicine. Note that a “generation” in the presence of colchicine consists of a normal cell cycle’s chromosomal duplication but no cell division. The two progeny cells were allowed to continue to grow, proceeding through the “cell cycle,” until each cell contained a set of metaphase chromosomes that looked like the following.



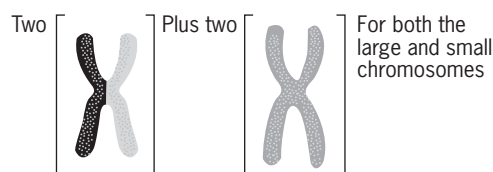
If autoradiography were carried out on these metaphase chromosomes (four large plus four small), what pattern of radioactivity (as indicated by silver grains on the autoradiograph) would be expected? (Assume no recombination between DNA molecules.)

**ANS:**



**10.8** Suppose that the experiment described in Problem 10.7 was carried out again, except this time replacing the  $^3\text{H}$ -thymidine with nonradioactive thymidine at the same time that the colchicine was added (after one cell generation of growth in  $^3\text{H}$ -thymidine-containing medium). The cells were then maintained in colchicine plus nonradioactive thymidine until the metaphase shown in Problem 10.7 occurred. What would the autoradiographs of these chromosomes look like?

**ANS:**



**10.9** Suppose that the DNA of cells (growing in a cell culture) in a eukaryotic species was labeled for a short period of time by the addition of  $^3\text{H}$ -thymidine to the medium. Next assume that the label was removed and the cells were resuspended in nonradioactive medium. After a short period of growth in nonradioactive medium, the DNA was extracted from these cells, diluted, gently layered on filters, and autoradiographed. If autoradiographs of the type

..... were observed, what would this indicate about the nature of DNA replication in these cells? Why?

**ANS:** The DNA replication was unidirectional rather than bidirectional. As the intracellular pools of radioactive  $^3\text{H}$ -thymidine are gradually diluted after transfer to nonradioactive medium, less and less  $^3\text{H}$ -thymidine will be incorporated into DNA at each replicating fork. This will produce autoradiograms with tails of decreasing grain density at each growing point. Since such tails appear at only one end of each track, replication must be unidirectional. Bidirectional replication would produce such tails at both ends of an autoradiographic track (see Figure 10.31).

**10.10** Arrange the following enzymes in the order of their action during DNA replication in *E. coli*: (1) DNA polymerase I, (2) DNA polymerase III, (3) DNA primase, (4) DNA gyrase, and (5) DNA helicase.

**ANS:** The correct sequence of action is 4, 5, 3, 2, 1.

**10.11** Fifteen distinct DNA polymerases— $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\epsilon$ ,  $\kappa$ ,  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\lambda$ ,  $\mu$ ,  $\sigma$ ,  $\phi$ , and Rev1—have been characterized in mammals. What are the intracellular locations and functions of these polymerases?

**ANS:** Current evidence suggests that polymerases  $\alpha$ ,  $\delta$ , and/or  $\epsilon$  are required for the replication of nuclear DNA. Polymerase  $\epsilon$  is thought to catalyze the continuous synthesis of the leading strand, and polymerases  $\alpha$  and  $\delta$  are thought to catalyze the replication of the lagging strand. Polymerase  $\alpha$  forms a complex with primase and initiates the synthesis of Okazaki fragments during the discontinuous replication of the lagging strand. Polymerase  $\alpha$  catalyzes the incorporation of approximately the first 30 nucleotides in each Okazaki fragment before being replaced by polymerase  $\delta$ , which then completes the synthesis of the fragments. Polymerase  $\gamma$  catalyzes replication of organellar chromosomes. Polymerases  $\beta$ ,  $\kappa$ ,  $\zeta$ ,  $\eta$ ,  $\theta$ ,  $\lambda$ ,  $\mu$ ,  $\sigma$ ,  $\phi$ , and Rev1 function in various DNA repair pathways (see Chapter 13).

**10.12** The *E. coli* chromosome contains approximately  $4 \times 10^6$  nucleotide pairs and replicates as a single bidirectional replicon in approximately 40 minutes under a wide variety of growth conditions. The largest chromosome of *D. melanogaster* contains about  $6 \times 10^7$  nucleotide pairs. (a) If this chromosome contains one giant molecule of

DNA that replicates bidirectionally from a single origin located precisely in the middle of the DNA molecule, how long would it take to replicate the entire chromosome if replication in *Drosophila* occurred at the same rate as replication in *E. coli*? (b) Actually, replication rates are slower in eukaryotes than in prokaryotes. If each replication bubble grows at a rate of 5000 nucleotide pairs per minute in *Drosophila* and 100,000 nucleotide pairs per minute in *E. coli*, how long will it take to replicate the largest *Drosophila* chromosome if it contains a single bidirectional replicon as described in (a) above? (c) In *Drosophila* embryos, the nuclei divide every 9 to 10 minutes. Based on your calculations in (a) and (b) earlier, what do these rapid nuclear divisions indicate about the number of replicons per chromosome in *Drosophila*?

**ANS:** (a) Given bidirectional replication of a single replicon, each replication fork must traverse  $2 \times 10^6$  nucleotide pairs in *E. coli* and  $3 \times 10^7$  nucleotide pairs in the largest *Drosophila* chromosome. If the rates were the same in both species, it would take 15 times ( $3 \times 10^7 / 2 \times 10^6$ ) as long to replicate the *Drosophila* chromosome or 10 hours (40 minutes  $\times 15 = 600$  minutes). (b) If replication forks in *E. coli* move 20 times as fast as replication forks in *Drosophila* (100,000 nucleotide pairs per minute/5000 nucleotide pairs per minute), the largest *Drosophila* chromosome would require 8.3 days (10 hours  $\times 20 = 200$  hours) to complete one round of replication. (c) Each *Drosophila* chromosome must contain many replicons in order to complete replication in less than 10 minutes.

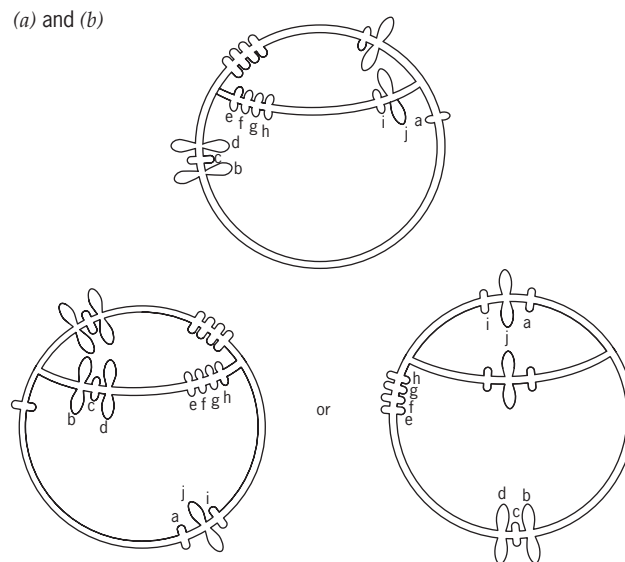
**10.13** *E. coli* cells that have been growing in  $^{14}\text{N}$  for many generations are transferred to medium containing only  $^{15}\text{N}$  and allowed to grow in this medium for four generations. Their DNA is then extracted and analyzed by equilibrium CsCl density-gradient centrifugation. What proportion of this DNA will band at the “light,” “hybrid,” and “heavy” positions in the gradient?

**ANS:** No DNA will band at the “light” position; 12.5 percent (2 of 16 DNA molecules) will band at the “hybrid” density; and 87.5 percent (14 of 16 DNA molecules) will band at the “heavy” position.

**10.14** The bacteriophage lambda chromosome has several A:T-rich segments that denature when exposed to pH 11.05 for 10 minutes. After such partial denaturation, the linear packaged form of the lambda DNA molecule has the structure shown in Figure 10.9a. Following its injection into an *E. coli* cell, the lambda DNA molecule is converted into a covalently closed circular molecule by hydrogen bonding between its complementary single-stranded termini and the action of DNA ligase. It then replicates as a  $\theta$ -shaped structure. The entire lambda chromosome is 17.5  $\mu\text{m}$  long. It has a unique origin of replication located 14.3  $\mu\text{m}$  from the left end of the linear form shown in Figure 10.9a. Draw the structure that would be observed by electron microscopy after both (1) replication of an approximately 6- $\mu\text{m}$ -long segment

of the lambda chromosomal DNA molecule (*in vivo*) and (2) exposure of this partially replicated DNA molecule to pH 11.05 for 10 minutes (*in vitro*), (a) if replication had proceeded bidirectionally from the origin, and (b) if replication had proceeded unidirectionally from the origin.

**ANS:**



**10.15** What enzyme activity catalyzes each of the following steps in the semiconservative replication of DNA in prokaryotes?

(a) The formation of negative supercoils in progeny DNA molecules. (b) The synthesis of RNA primers. (c) The removal of RNA primers. (d) The covalent extension of DNA chains at the 3'-OH termini of primer strands. (e) Proofreading of the nucleotides at the 3'-OH termini of DNA primer strands?

**ANS:** (a) DNA gyrase; (b) primase; (c) the 5'  $\rightarrow$  3' exonuclease activity of DNA polymerase I; (d) the 5'  $\rightarrow$  3' polymerase activity of DNA polymerase III; (e) the 3'  $\rightarrow$  5' exonuclease activity of DNA polymerase III.

**10.16** One species of tree has a very large genome consisting of  $2.0 \times 10^{10}$  base pairs of DNA.

(a) If this DNA was organized into a single linear molecule, how long (meters) would this molecule be? (b) If the DNA is evenly distributed among 10 chromosomes and each chromosome has one origin of DNA replication, how long would it take to complete the S phase of the cell cycle, assuming that DNA polymerase can synthesize  $2 \times 10^4$  bp of DNA per minute? (c) An actively growing cell can complete the S phase of the cell cycle in approximately 300 minutes. Assuming that the origins of replication are evenly distributed, how many origins of replication are present on each chromosome? (d) What is the average number of base pairs between adjacent origins of replication?

**ANS:** (a)  $(34 \text{ nm}/100 \text{ bp})(2 \times 10^{10} \text{ bp}) = 6.8 \times 10^9 = 6.8 \text{ meters}$ .  
 (b)  $2 \times 10^{10}/10 \text{ chromosomes} = 2 \times 10^9 \text{ bp}$ ;  $4 \times 10^4 \text{ bp}/\text{min}$  (bidirectional);  $2 \times 10^9/4 \times 10^4 = 5 \times 10^4 \text{ min} = 50,000 \text{ min}$ . (c)  $(50,000 \text{ min})(1 \text{ ori}) = (300 \text{ min})(X \text{ ori})$ ;  $X = 50,000/300 = 167 \text{ ori}$ . (d)  $(2 \times 10^9 \text{ bp}/\text{chrom.})/(167 \text{ ori}/\text{chrom.}) = 1.2 \times 10^7 \text{ bp/ori}$ .

**10.17** Why must each of the giant DNA molecules in eukaryotic chromosomes contain multiple origins of replication?

**ANS:** In eukaryotes, the rate of DNA synthesis at each replication fork is about 2500–3000 nucleotide pairs per minute. Large eukaryotic chromosomes often contain  $10^7$ – $10^8$  nucleotide pairs. A single replication fork could not replicate the giant DNA in one of these large chromosomes fast enough to permit the observed cell generation times.

**10.18** In *E. coli*, viable *polA* mutants have been isolated that produce a defective gene product with little or no 5'→3' polymerase activity, but normal 5'→3' exonuclease activity. However, no *polA* mutant has been identified that is completely deficient in the 5'→3' exonuclease activity, while retaining 5'→3' polymerase activity, of DNA polymerase I. How can these results be explained?

**ANS:** The 5' → 3' exonuclease activity of DNA polymerase I is essential to the survival of the bacterium, whereas the 5'→3' polymerase activity of the enzyme is not essential.

**10.19** Other *polA* mutants of *E. coli* lack the 3' → 5' exonuclease activity of DNA polymerase I. Will the rate of DNA synthesis be altered in these mutants? What effect(s) will these *polA* mutations have on the phenotype of the organism?

**ANS:** No, the rate of DNA synthesis will not be altered. *E. coli* strains carrying *polA* mutations that eliminate the 3' → 5' exonuclease activity of DNA polymerase I will exhibit unusually high mutation rates.

**10.20** Many of the origins of replication that have been characterized contain AT-rich core sequences. Are these AT-rich cores of any functional significance? If so, what?

**ANS:** Because AT base pairs are held together by only two hydrogen bonds instead of the three hydrogen bonds present in GC base pairs, the two strands of AT-rich regions of double helices are separated more easily, providing the single-stranded template regions required for DNA replication.

**10.21** (a) Why isn't DNA primase activity required to initiate rolling-circle replication?(b) DNA primase is required for the discontinuous synthesis of the lagging strand, which occurs on the single-stranded tail of the rolling circle. Why?

**ANS:** Rolling-circle replication begins when an endonuclease cleaves one strand of a circular DNA double helix. This cleavage produces a free 3'-OH on one end of the cut

strand, allowing it to function as a primer. The discontinuous synthesis of the lagging strand requires the *de novo* initiation of each Okazaki fragment, which requires DNA primase activity.

**10.22** DNA polymerase I is needed to remove RNA primers during chromosome replication in *E. coli*. However, DNA polymerase III is the true replicase in *E. coli*. Why does not DNA polymerase III remove the RNA primers?

**ANS:** DNA polymerase III does not have a 5' → 3' exonuclease activity that acts on double-stranded nucleic acids. Thus, it cannot excise RNA primer strands from replicating DNA molecules. DNA polymerase I is present in cells at much higher concentrations and functions as a monomer. Thus, DNA polymerase I is able to catalyze the removal of RNA primers from the vast number of Okazaki fragments formed during the discontinuous replication of the lagging strand.

**10.23** In *E. coli*, three different proteins are required to unwind the parental double helix and keep the unwound strands in an extended template form. What are these proteins, and what are their respective functions?

**ANS:** DNA helicase unwinds the DNA double helix, and single-strand DNA-binding protein coats the unwound strands, keeping them in an extended state. DNA gyrase catalyzes the formation of negative supercoiling in *E. coli* DNA, and this negative supercoiling behind the replication forks is thought to drive the unwinding process because superhelical tension is reduced by unwinding the complementary strands.

**10.24** How similar are the structures of DNA polymerase I and DNA polymerase III in *E. coli*? What is the structure of the DNA polymerase III holoenzyme? What is the function of the *dnaN* gene product in *E. coli*?

**ANS:** DNA polymerase I is a single polypeptide of molecular weight 109,000, whereas DNA polymerase III is a complex multimeric protein. The DNA polymerase III holoenzyme has a molecular mass of about 900,000 daltons and is composed of at least 20 different polypeptides. The *dnaN* gene product, the β subunit of DNA polymerase III, forms a dimeric clamp that encircles the DNA molecule and prevents the enzyme from dissociating from the template DNA during replication.

**10.25** The *dnaA* gene product of *E. coli* is required for the initiation of DNA synthesis at *oriC*. What is its function? How do we know that the DnaA protein is essential to the initiation process?

**ANS:** DnaA protein initiates the formation of the replication bubble by binding to the 9-bp repeats of *OriC*. DnaA protein is known to be required for the initiation process because bacteria with temperature-sensitive mutations in the *dnaA* gene cannot initiate DNA replication at restrictive temperatures.

**10.26** What is a primosome, and what are its functions? What essential enzymes are present in the primosome? What are the major components of the *E. coli* replisome? How can geneticists determine whether these components are required for DNA replication?

**ANS:** The primosome is a protein complex that initiates the synthesis of Okazaki fragments during lagging strand synthesis. The major components of the *E. coli* DNA primosome are DNA primase and DNA helicase. Geneticists have been able to show that both DNA primase and DNA helicase are required for DNA replication by demonstrating that mutations in the genes encoding these enzymes result in the arrest of DNA synthesis in mutant cells under conditions where the altered proteins are inactive.

**10.27** The chromosomal DNA of eukaryotes is packaged into nucleosomes during the S phase of the cell cycle. What obstacles do the size and complexity of both the replisome and the nucleosome present during the semiconservative replication of eukaryotic DNA? How might these obstacles be overcome?

**ANS:** Nucleosomes and replisomes are both large macromolecular structures, and the packaging of eukaryotic DNA into nucleosomes raises the question of how a replisome can move past a nucleosome and replicate the DNA in the nucleosome in the process. The most obvious solution to this problem would be to completely or partially disassemble the nucleosome to allow the replisome to pass. The nucleosome would then reassemble after the replisome had passed. One popular model has the nucleosome partially disassembling, allowing the replisome to move past it (see Figure 10.33b).

**10.28** Two mutant strains of *E. coli* each have a temperature-sensitive mutation in a gene that encodes a product required for chromosome duplication. Both strains replicate their DNA and divide normally at 25°C but are unable to replicate their DNA or divide at 42°C. When cells of one strain are shifted from growth at 25°C to growth at 42°C, DNA synthesis stops immediately. When cells of the other strain are subjected to the same temperature shift, DNA synthesis continues, albeit at a decreasing rate, for about a half hour. What can you conclude about the functions of the products of these two genes?

**ANS:** The product of the first gene is required for DNA chain extension, whereas the product of the second gene is only required for the initiation of DNA synthesis.

**10.29** In what ways does chromosomal DNA replication in eukaryotes differ from DNA replication in prokaryotes?

**ANS:** (1) DNA replication usually occurs continuously in rapidly growing prokaryotic cells but is restricted to the S phase of the cell cycle in eukaryotes. (2) Most eukaryotic chromosomes contain multiple origins of replication, whereas most prokaryotic chromosomes

contain a single origin of replication. (3) Prokaryotes utilize two catalytic complexes that contain the same DNA polymerase to replicate the leading and lagging strands, whereas eukaryotes utilize two or three distinct DNA polymerases for leading and lagging strand synthesis. (4) Replication of eukaryotic chromosomes requires the partial disassembly and reassembly of nucleosomes as replisomes move along parental DNA molecules. In prokaryotes, replication probably involves a similar partial disassembly/reassembly of nucleosome-like structures. (5) Most prokaryotic chromosomes are circular and thus have no ends. Most eukaryotic chromosomes are linear and have unique termini called telomeres that are added to replicating DNA molecules by a unique, RNA-containing enzyme called telomerase.

**10.30** (a) The chromosome of the bacterium *Salmonella typhimurium* contains about  $4 \times 10^6$  nucleotide pairs. Approximately how many Okazaki fragments are produced during one complete replication of the *S. typhimurium* chromosome? (b) The largest chromosome of *D. melanogaster* contains approximately  $6 \times 10^7$  nucleotide pairs. About how many Okazaki fragments are produced during the replication of this chromosome?

**ANS:** (a) 2000–4000 Okazaki fragments. (b) 300,000–600,000 Okazaki fragments.

**10.31** In the yeast *S. cerevisiae*, haploid cells carrying a mutation called *est1* (for *ever-shorter telomeres*) lose distal telomere sequences during each cell division. Predict the ultimate phenotypic effect of this mutation on the progeny of these cells.

**ANS:** The chromosomes of haploid yeast cells that carry the *est1* mutation become shorter during each cell division. Eventually, chromosome instability results from the complete loss of telomeres, and cell death occurs because of the deletion of essential genes near the ends of chromosomes.

**10.32** Assume that the sequence of a double-stranded DNA shown below is present at one end of a large DNA molecule in a eukaryotic chromosome.

5'-(centromere sequence)-gattccccgggaagcttggggggcccatcttctgtagctttgca-3'  
3'-(centromere sequence)-ctaaggggccccttgaacccccgggtagaagcatgagaacgt-5'

You have reconstituted a eukaryotic replisome that is active *in vitro*. However, it lacks telomerase activity. If you isolate the DNA molecule shown above and replicate it in your *in vitro* system, what products would you expect?

**ANS:** Without telomerase, the 5' end of the newly replicated strand will be missing some bases. The exact number of missing bases does not matter:

5'-(centromere sequence)-gattccccgggaagcttggggggcccatcttctgtagctttgca-3'  
3'-(centromere sequence)-ctaaggggccccttgaacccccgggtagaagcatg-5'  
5'-(centromere sequence)-gattccccgggaagcttggggggcccatcttctgtagctttgca-3'  
3'-(centromere sequence)-ctaaggggccccttc-5'

## CHAPTER 11

**11.1** Distinguish between DNA and RNA (a) chemically, (b) functionally, and (c) by location in the cell.

**ANS:** (a) RNA contains the sugar ribose, which has a hydroxyl (OH) group on the 2-carbon; DNA contains the sugar 2-deoxyribose, with only hydrogens on the 2-carbon. RNA usually contains the base uracil at positions where thymine is present in DNA. However, some DNAs contain uracil, and some RNAs contain thymine. DNA exists most frequently as a double helix (double-stranded molecule); RNA exists more frequently as a single-stranded molecule. But, some DNAs are single-stranded and some RNAs are double-stranded. (b) The main function of DNA is to store genetic information and to transmit that information from cell to cell and from generation to generation. RNA stores and transmits genetic information in some viruses that contain no DNA. In cells with both DNA and RNA: (1) mRNA acts as an intermediary in protein synthesis, carrying the information from DNA in the chromosomes to the ribosomes (sites at which proteins are synthesized). (2) tRNAs carry amino acids to the ribosomes and function in codon recognition during the synthesis of polypeptides. (3) rRNA molecules are essential components of the ribosomes. (4) snRNAs are important components of spliceosomes. (5) miRNAs play key roles in regulating gene expression (see Chapter 18). (c) DNA is located primarily in the chromosomes, which are found in the nucleus of eukaryotic cells; however, some DNA is also found in cytoplasmic organelles, such as mitochondria and chloroplasts. RNA is located throughout cells.

**11.2** What bases in the mRNA transcript would represent the following DNA template sequence: 5'-TGCAGACA-3'?

**ANS:** 3'-ACGUCUGU-5'

**11.3** What bases in the transcribed strand of DNA would give rise to the following mRNA base sequence: 5'-CUGAU-3'?

**ANS:** 3'-GACTA-5'

**11.4** On the basis of what evidence was the messenger RNA hypothesis established?

**ANS:** The genetic information of cells is stored in DNA, which is located predominantly in the chromosomes. The gene products (polypeptides) are synthesized primarily in the cytoplasm on ribosomes. Some intermediate must therefore carry the genetic information from the chromosomes to the ribosomes. RNA molecules (mRNAs) were shown to perform this function by means of RNA pulse-labeling and pulse-chase experiments combined with autoradiography. The enzyme RNA polymerase was subsequently shown to catalyze the synthesis of mRNA using chromosomal DNA as a template. Finally, the mRNA molecules synthesized by RNA polymerase were shown to faithfully direct the synthesis of specific polypeptides when used in *in vitro* protein synthesis systems.

**11.5** At what locations in a eukaryotic cell does protein synthesis occur?

**ANS:** Protein synthesis occurs on ribosomes. In eukaryotes, most of the ribosomes are located in the cytoplasm and are attached to the extensive membranous network of endoplasmic reticulum. Some protein synthesis also occurs in cytoplasmic organelles such as chloroplasts and mitochondria.

**11.6** List three ways in which the mRNAs of eukaryotes differ from the mRNAs of prokaryotes.

**ANS:** (1) Eukaryotes have a 5' cap on their mRNAs; prokaryotes do not. (2) Messenger RNAs of eukaryotes generally have a 3' poly-A tail, prokaryotic mRNAs do not. (3) Messenger RNA formation in eukaryotes involves removal of introns (when present) and the splicing together of exons. Prokaryotic genes (with very rare exceptions) do not have introns.

**11.7** What different types of RNA molecules are present in prokaryotic cells? in eukaryotic cells? What roles do these different classes of RNA molecules play in the cell?

**ANS:** Both prokaryotic and eukaryotic organisms contain messenger RNAs, transfer RNAs, and ribosomal RNAs. In addition, eukaryotes contain small nuclear RNAs and micro RNAs. Messenger RNA molecules carry genetic information from the chromosomes (where the information is stored) to the ribosomes in the cytoplasm (where the information is expressed during protein synthesis). The linear sequence of triplet codons in an mRNA molecule specifies the linear sequence of amino acids in the polypeptides produced during translation of that mRNA. Transfer RNA molecules are small (about 80 nucleotides long) molecules that carry amino acids to the ribosomes and provide the codon-recognition specificity during translation. Ribosomal RNA molecules provide part of the structure and function of ribosomes; they represent an important part of the machinery required for the synthesis of polypeptides. Small nuclear RNAs are structural components of spliceosomes, which excise noncoding intron sequences from nuclear gene transcripts. Micro RNAs are involved in the regulation of gene expression.

**11.8** Many eukaryotic genes contain noncoding introns that separate the coding sequences or exons of these genes. At what stage during the expression of these split genes are the noncoding intron sequences removed?

**ANS:** The entire nucleotide-pair sequences—including the introns—of the genes are transcribed by RNA polymerase to produce primary transcripts that still contain the intron sequences. The intron sequences are then spliced out of the primary transcripts to produce the mature, functional RNA molecules. In the case of protein-encoding nuclear genes of higher eukaryotes, the introns are spliced out by complex macromolecular structures called spliceosomes.

**11.9** For several decades, the dogma in biology has been that molecular reactions in living cells are catalyzed by enzymes composed of polypeptides. We now know that



the introns of some precursor RNA molecules such as the rRNA precursors in *Tetrahymena* are removed autocatalytically (self-spliced) with no involvement of any catalytic protein. What does the demonstration of autocatalytic splicing indicate about the dogma that biological reactions are always catalyzed by proteinaceous enzymes?

**ANS:** “Self-splicing” of RNA precursors demonstrates that RNA molecules can also contain catalytic sites; this property is not restricted to proteins.

**11.10** What role(s) do spliceosomes play in pathways of gene expression? What is their macromolecular structure?

**ANS:** Spliceosomes excise intron sequences from nuclear gene transcripts to produce the mature mRNA molecules that are translated on ribosomes in the cytoplasm. Spliceosomes are complex macromolecular structures composed of snRNA and protein molecules (see Figure 11.22).

**11.11** What components of the introns of nuclear genes that encode proteins in higher eukaryotes are conserved and required for the correct excision of intron sequences from primary transcripts by spliceosomes?

**ANS:** The introns of protein-encoding nuclear genes of higher eukaryotes almost invariably begin (5′) with GT and end (3′) with AG. In addition, the 3′ subterminal A in the “TACTAAC box” is completely conserved; this A is involved in bond formation during intron excision.

**11.12** Match one of the following terms with each of the descriptions given below. *Terms:* (1) sigma ( $\sigma$ ) factor; (2) poly(A) tail; (3) TATAAT; (4) exons; (5) TATAAAA; (6) RNA polymerase III; (7) intron; (8) RNA polymerase II; (9) heterogeneous nuclear RNA (hnRNA); (10) snRNA; (11) RNA polymerase I; (12) TTGACA; (13) GGCCAATCT (CAAT box).

*Descriptions:*

(a) Intervening sequence found in many eukaryotic genes.

(b) A conserved nucleotide sequence (−30) in eukaryotic promoters involved in the initiation of transcription.

(c) Small RNA molecules that are located in the nuclei of eukaryotic cells, most as components of the spliceosome, that participate in the excision of introns from nuclear gene transcripts.

(d) A sequence (−10) in the nontemplate strand of the promoter of *E. coli* that facilitates the localized unwinding of DNA when complexed with RNA polymerase.

(e) The RNA polymerase in the nucleus that catalyzes the synthesis of all rRNAs except for the small 5S rRNA.

(f) The subunit of prokaryotic RNA polymerase that is responsible for the initiation of transcription at promoters.

(g) An *E. coli* promoter sequence located 35 nucleotides upstream from the transcription-initiation site; it serves as a recognition site for the sigma factor.

(h) The RNA polymerase in the nucleus that catalyzes the synthesis of the transfer RNA molecules and small nuclear RNAs.

(i) A polyadenosine tract 20–200 nucleotides long that is added to the 3′ end of most eukaryotic messenger RNAs.

(j) The RNA polymerase that transcribes nuclear genes that encode proteins.

(k) A conserved sequence in the nontemplate strand of eukaryotic promoters that is located about 80 nucleotides upstream from the transcription start site.

(l) Segments of an eukaryotic gene that correspond to the sequences in the final processed RNA transcript of the gene.

(m) The population of primary transcripts in the nucleus of a eukaryotic cell.

**ANS:** (a) 7; (b) 5; (c) 10; (d) 3; (e) 11; (f) 1; (g) 12; (h) 6; (i) 2; (j) 8; (k) 13; (l) 4; (m) 9.

**11.13** (a) Which of the following nuclear pre-mRNA nucleotide sequences potentially contains an intron?

(1) 5′-UGACCAUGGCGCUAACACUGCCAAUUGGCAAUACUGACCUGAUAGCAUCAGCCAA-3′

(2) 5′-UAGUCUCAUCUGUCCAUUGACUUCGAAA CUGAAUCGUAACUCCUACGUCUAUGGA-3′

(3) 5′-UAGCUGUUUGUCAUGACUGACUGGUCA CUAUCGUACUAACCUGUCAUGCAAUGUC-3′

(4) 5′-UAGCAGUUCUGUCGCCUCGUGGUCGUCUGGCCCUUCGUCGUCGCGGGCUUAGCUA-3′

(5) 5′-UAGGUUCGCAUUGACGUACUUCUGAAACUACUAACUACUAACGCAUCGAGUCUCAA-3′

(b) One of the five pre-mRNAs shown in (a) may undergo RNA splicing to excise an intron sequence. What mRNA nucleotide sequence would be expected to result from this splicing event?

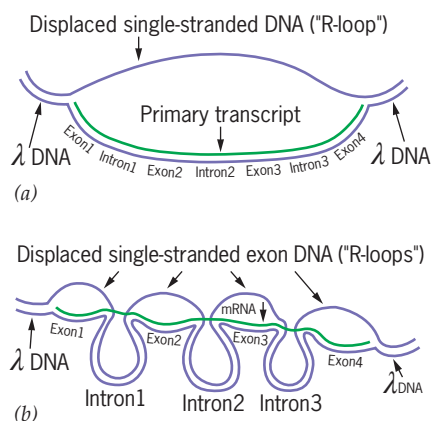
**ANS:** (a) Sequence 5. It contains the conserved intron sequences: a 5′ GU, a 3′ AG, and a UACUAA internal sequence providing a potential bonding site for intron excision. Sequence 4 has a 5′ GU and a 3′ AG but contains no internal A for the bonding site during intron excision. (b) 5′—UAGUCUCAA—3′; the putative intron from the 5′ GU through the 3′ AG has been removed.

**11.14** What is the function of the introns in eukaryotic genes?

**ANS:** This is a wide-open question at present! There is much speculation, but little hard evidence. One popular hypothesis is that introns enhance exon shuffling by increasing recombination events between sequences encoding adjacent domains of a polypeptide. Also, in one yeast mitochondrial gene, the introns contain open reading frames that encode “maturases” that splice out these introns—a neat negative feedback control. Other introns may be merely relics of evolution.

- 11.15** A particular gene is inserted into the phage lambda chromosome and is shown to contain three introns. (a) The primary transcript of this gene is purified from isolated nuclei. When this primary transcript is hybridized under R-loop conditions with the recombinant lambda chromosome carrying the gene, what will the R-loop structure(s) look like? Label your diagram. (b) The mRNA produced from the primary transcript of this gene is then isolated from cytoplasmic polyribosomes and similarly examined by the R-loop hybridization procedure using the recombinant lambda chromosome carrying the gene. Diagram what the R-loop structure(s) will look like when the cytoplasmic mRNA is used. Again, label the components of your diagram.

**ANS:**



- 11.16** A segment of DNA in *E. coli* has the following sequence of nucleotide pairs:

```
3'-A TGCTACTGCTATTCGCTGTATCG-5'
  |||
5'-TACGATGACGATAAGCGACATAGC-3'
```

When this segment of DNA is transcribed by RNA polymerase, what will be the sequence of nucleotides in the RNA transcript if the promoter is located to the left of the sequence shown?

- ANS:** If there is a promoter located upstream from this DNA segment, the nucleotide sequence of this portion of the RNA transcript will be

5'-UACGAUGACGAUAAGCGACAUAAGC-3'. If there is no upstream promoter, this segment of DNA will not be transcribed.

- 11.17** A segment of DNA in *E. coli* has the following sequence of nucleotide pairs:

```
3'-A TATTACTGCAATGGGCTGTATCG-
  |||
5'-TATAATGACGTTACCCGACATAGC-

ATGCTACTGCTATTCGCTGTATCG-5'
  |||
TACGATGACGATAAGCGACATAGC-3'
```

When this segment of DNA is transcribed by RNA polymerase, what will be the sequence of nucleotides in the RNA transcript?

- ANS:** Assuming that there is a  $-35$  sequence upstream from the consensus  $-10$  sequence in this segment of the DNA molecule, the nucleotide sequence of the transcript will be 5'-ACCCGACAUAGCUACGAUGACGAUAAGC GACAUAAGC-3'.

- 11.18** A segment of DNA in *E. coli* has the following sequence of nucleotide pairs:

```
3'-AACTGTACGTGCTACCTTGCTGATATTACT-
  |||
5'-TTGACATGCACGATGGAACGACTATAATGA-

GCAATGGGCTGTATCGATGCTACTGCTAT-5'
  |||
CGTTACCCGACATAGCTACGATGACGATA-3'
```

When this segment of DNA is transcribed by RNA polymerase, what will be the sequence of nucleotides in the RNA transcript?

- ANS:** Given the consensus  $-35$  and  $-10$  sequences in this segment of DNA and the fact that transcripts almost always start with a purine, the predicted nucleotide sequence of the transcript is 5'-ACCCGACAUAGCUACGAUGA CGAUA-3'.

- 11.19** A segment of human DNA has the following sequence of nucleotide pairs:

```
3'-ATATTTACGTGCTACCTTGCTGATAGGACT-
  |||
5'-TATAAATGCACGATGGAACGACTATCCTGA-

GCAATGGGCTGTATCGATGCTACTGCTAT-5'
  |||
CGTTACCCGACATAGCTACGATGACGATA-3'
```

When this segment of DNA is transcribed by RNA polymerase, what will be the sequence of nucleotides in the RNA transcript?

- ANS:** Assuming that there is a CAAT box located upstream from the TATA box shown in this segment of DNA, the nucleotide sequence of the transcript will be 5'-ACCCGACAUAGCUACGAUGACGAUA-3'.

- 11.20** The genome of a human must store a tremendous amount of information using the four nucleotide pairs present in DNA. What does the language of computers tell us about the feasibility of storing large amounts of information using an alphabet composed of just four letters?

- ANS:** Given the vast amount of information that can be stored on a small computer chip by using a binary code, it is clear that large quantities of genetic information can be stored in the genomes of organisms by using the four-letter alphabet of the genetic code.

- 11.21** What is the central dogma of molecular genetics? What impact did the discovery of RNA tumor viruses have on the central dogma?

**ANS:** According to the central dogma, genetic information is stored in DNA and is transferred from DNA to RNA to protein during gene expression. RNA tumor viruses store their genetic information in RNA, and that information is copied into DNA by the enzyme reverse transcriptase after a virus infects a host cell. Thus, the discovery of RNA tumor viruses or retroviruses—retro for backwards flow of genetic information—provided an exception to the central dogma.

**11.22** The biosynthesis of metabolite X occurs via six steps catalyzed by six different enzymes. What is the minimal number of genes required for the genetic control of this metabolic pathway? Might more genes be involved? Why?

**ANS:** If six different enzymes are required for the pathway, then minimally six genes are necessary for genetic control of the pathway. However, because the expression of these enzymes may rely on the product of other genes, such as transcription factors, more than six genes could be involved in genetic control of this pathway.

**11.23** What do processes of DNA synthesis, RNA synthesis, and polypeptide synthesis have in common?

**ANS:** DNA, RNA, and protein synthesis all involve the synthesis of long chains of repeating subunits. All three processes can be divided into three stages: chain initiation, chain elongation, and chain termination.

**11.24** What are the two stages of gene expression? Where do they occur in a eukaryotic cell? a prokaryotic cell?

**ANS:** The two stages of gene expression are as follows:

(1) Transcription—the transfer of genetic information from DNA to RNA.

(2) Translation—the transfer of genetic information from RNA to protein. In eukaryotes, transcription occurs in the nucleus and translation occurs in the cytoplasm on complex macromolecular structures called ribosomes. In prokaryotes, transcription and translation are often coupled with mRNA molecules often being translated by ribosomes while still being synthesized during transcription.

**11.25** Compare the structures of primary transcripts with those of mRNAs in prokaryotes and eukaryotes. On average, in which group of organisms do they differ the most?

**ANS:** The primary transcripts of eukaryotes undergo more extensive posttranscriptional processing than those of prokaryotes. Thus, the largest differences between mRNAs and primary transcripts occur in eukaryotes. Transcript processing is usually restricted to the excision of terminal sequences in prokaryotes. In contrast, eukaryotic transcripts are usually modified by (1) the excision of intron sequences; (2) the addition of 7-methyl guanosine caps to the 5' termini; (3) the addition of poly(A) tails to the 3' termini. In addition, the sequences of some eukaryotic transcripts are modified by RNA editing processes.

**11.26** What five types of RNA molecules participate in the process of gene expression? What are the functions of each type of RNA? Which types of RNA perform their function(s) in (a) the nucleus and (b) the cytoplasm?

**ANS:** The five types of RNA molecules that are involved in gene expression are mRNAs, rRNAs, tRNAs, micro RNAs, and snRNAs. mRNA molecules carry genetic information from genes to the sites of protein synthesis and specify the amino acid sequences of polypeptides. rRNAs are major structural components of the ribosomes and provide functions required for translation. tRNA molecules are the adapters that provide amino acid-codon specificity during translation; each tRNA is activated by a specific amino acid and contains an anticodon sequence that is complementary or partially complementary to one, two, or three codons in mRNAs. Micro-RNAs are involved in regulative gene expression (see Chapter 18). snRNAs are structural components of the spliceosomes that excise introns from gene transcripts in eukaryotes. snRNAs perform their splicing functions in the nucleus. mRNAs carry information from the nucleus to the cytoplasm, so they function in both compartments of the cell. However, their most prominent function is to direct the synthesis of polypeptides during translation, which occurs in the cytoplasm. rRNAs and tRNAs perform their functions during translation in the cytoplasm. Micro-RNAs become incorporated into ribonucleoprotein complexes in the cytoplasm.

**11.27** Why was the need for an RNA intermediary in protein synthesis most obvious in eukaryotes? How did researchers first demonstrate that RNA synthesis occurred in the nucleus and that protein synthesis occurred in the cytoplasm?

**ANS:** In eukaryotes, the genetic information is stored in DNA in the nucleus, whereas proteins are synthesized on ribosomes in the cytoplasm. How could the genes, which are separated from the sites of protein synthesis by a double-membrane—the nuclear envelope, direct the synthesis of polypeptides without some kind of intermediary to carry the specifications for the polypeptides from the nucleus to the cytoplasm? Researchers first used labeled RNA and protein precursors and autoradiography to demonstrate that RNA synthesis and protein synthesis occurred in the nucleus and the cytoplasm, respectively.

**11.28** Two eukaryotic genes encode two different polypeptides, each of which is 335 amino acids long. One gene contains a single exon; the other gene contains an intron of 41,324 nucleotide pairs long. Which gene would you expect to be transcribed in the least amount of time? Why? When the mRNAs specified by these genes are translated, which mRNA would you expect to be translated in the least time? Why?

**ANS:** Because transcription results in a primary transcript from the DNA template, the DNA sequence for the single-exon gene is the shortest, and so it will be transcribed in

the least amount of time. However, because each polypeptide is the same length, the mature mRNAs for both genes will be the same length and will be translated in the same amount of time.

**11.29** Design an experiment to demonstrate that RNA transcripts are synthesized in the nucleus of eukaryotes and are subsequently transported to the cytoplasm.

**ANS:** A simple pulse- and pulse/chase-labeling experiment will demonstrate that RNA is synthesized in the nucleus and is subsequently transported to the cytoplasm. This experiment has two parts: (1) pulse label eukaryotic culture cells by growing them in  $^3\text{H}$ -uridine for a few minutes and localize the incorporated radioactivity by autoradiography. (2) Repeat the experiment, but this time add a large excess of nonradioactive uridine to the medium in which the cells are growing after the labeling period and allow the cells to grow in the nonradioactive medium for about an hour. Then localize the incorporated radioactivity by autoradiography.

**11.30** Total RNA was isolated from human cells growing in culture. This RNA was mixed with nontemplate strands (single strands) of the human gene encoding the enzyme thymidine kinase, and the RNA–DNA mixture was incubated for 12 hours under renaturation conditions. Would you expect any RNA–DNA duplexes to be formed during the incubation? If so, why? If not, why not? The same experiment was then performed using the template strand of the thymidine kinase gene. Would you expect any RNA–DNA duplexes to be formed in this second experiment? If so, why? If not, why not?

**ANS:** RNA–DNA duplexes will be formed when the template strand is used, but not when the nontemplate strand is used. (However, if some hybridization is observed with the nontemplate strand, this is because total RNA was used and is nonspecific to the thymidine kinase gene) Only one strand—the template strand—of most genes is transcribed. Thus, RNA will contain nucleotide sequences complementary to the template strand but not to the nontemplate strand.

**11.31** Two preparations of RNA polymerase from *E. coli* are used in separate experiments to catalyze RNA synthesis *in vitro* using a purified fragment of DNA carrying the *argH* gene as template DNA. One preparation catalyzes the synthesis of RNA chains that are highly heterogeneous in size. The other preparation catalyzes the synthesis of RNA chains that are all the same length. What is the most likely difference in the composition of the RNA polymerases in the two preparations?

**ANS:** The first preparation of RNA polymerase is probably lacking the sigma subunit and, as a result, initiates the synthesis of RNA chains at random sites along both strands of the *argH* DNA. The second preparation probably contains the sigma subunit and initiates RNA chains only at the site used *in vivo*, which is governed by the position of the  $-10$  and  $-35$  sequences of the promoter.

**11.32** Transcription and translation are coupled in prokaryotes. Why is this not the case in eukaryotes?

**ANS:** In eukaryotes, transcription occurs in the nucleus and translation occurs in the cytoplasm. Because these processes occur in different compartments of the cell, they cannot be coupled as they are in prokaryotes.

**11.33** What two elements are almost always present in the promoters of eukaryotic genes that are transcribed by RNA polymerase II? Where are these elements located relative to the transcription start site? What are their functions?

**ANS:** TATA and CAAT boxes. The TATA and CAAT boxes are usually centered at positions  $-30$  and  $-80$ , respectively, relative to the startpoint ( $+1$ ) of transcription. The TATA box is responsible for positioning the transcription startpoint; it is the binding site for the first basal transcription factor that interacts with the promoter. The CAAT box enhances the efficiency of transcriptional initiation.

**11.34** In what ways are most eukaryotic gene transcripts modified? What are the functions of these posttranscriptional modifications?

**ANS:** (1) Intron sequences are spliced out of gene transcripts to provide contiguous coding sequences for translation. (2) The 7-methyl guanosine caps added to the 5' termini of most eukaryotic mRNAs help protect them from degradation by nucleases and are recognized by proteins involved in the initiation of translation. (3) The poly(A) tails at the 3' termini of mRNAs play an important role in their transport from the nucleus to the cytoplasm and enhance their stability.

**11.35** How does RNA editing contribute to protein diversity in eukaryotes?

**ANS:** RNA editing sometimes leads to the synthesis of two or more distinct polypeptides from a single mRNA.

**11.36** How do the mechanisms by which the introns of tRNA precursors, *Tetrahymena* rRNA precursors, and nuclear pre-mRNAs are excised differ? In which process are snRNAs involved? What role(s) do these snRNAs play?

**ANS:** The introns of tRNA precursors, *Tetrahymena* rRNA precursors, and nuclear pre-mRNAs are excised by completely different mechanisms. (1) Introns in tRNA are excised by cleavage and joining events catalyzed by splicing nucleases and ligases, respectively. (2) Introns in *Tetrahymena* rRNA precursors are excised autocatalytically. (3) Introns of nuclear pre-mRNAs are excised by spliceosomes. snRNAs are involved in nuclear pre-mRNA splicing as structural components of spliceosomes. In addition, snRNA U1 is required for the cleavage events at the 5' termini of introns; U1 is thought to base-pair with a partially complementary consensus sequence at this position in pre-mRNAs.

**11.37** A mutation in an essential human gene changes the 5' splice site of a large intron from GT to CC. Predict the phenotype of an individual homozygous for this mutation.

**ANS:** This zygote will probably be nonviable because the gene product is essential, and the elimination of the 5' splice site will almost certainly result in the production of a nonfunctional gene product.

**11.38** Total RNA was isolated from nuclei of human cells growing in culture. This RNA was mixed with a purified, denatured DNA fragment that carried a large intron of a housekeeping gene (a gene expressed in essentially all cells), and the RNA–DNA mixture was incubated for 12 hours under renaturation conditions. Would you expect any RNA–DNA duplexes to be formed during the incubation? If so, why? If not, why not? The same experiment was then performed using total cytoplasmic RNA from these cells. Would you expect any RNA–DNA duplexes to be formed in this second experiment? If so, why? If not, why not?

**ANS:** In the first experiment, yes, some hybridization would be expected because not all of the RNA in the preparation has been completely processed and will still contain the intron sequence. However, if only cytoplasmic RNA is used in the hybridization experiment, all of the mRNA in the preparation will have been processed (because processing occurs in the nucleus) and no hybridization will be observed.

## CHAPTER 12

**12.1** In a general way, describe the molecular organization of proteins and distinguish proteins from DNA, chemically and functionally. Why is the synthesis of proteins of particular interest to geneticists?

**ANS:** Proteins are long chainlike molecules made up of amino acids linked together by peptide bonds. Proteins are composed of carbon, hydrogen, nitrogen, oxygen, and usually sulfur. They provide the enzymatic capacity and much of the structure of living organisms. DNA is composed of phosphate, the pentose sugar 2-deoxyribose, and four nitrogen-containing organic bases (adenine, cytosine, guanine, and thymine). DNA stores and transmits the genetic information in most living organisms. Protein synthesis is of particular interest to geneticists because proteins are the primary gene products—the key intermediates through which genes control the phenotypes of living organisms.

**12.2** At what locations in the cell does protein synthesis occur?

**ANS:** Protein synthesis occurs on ribosomes. In eukaryotes, most of the ribosomes are located in the cytoplasm and are attached to the extensive membranous network of endoplasmic reticulum. Some protein synthesis also occurs in cytoplasmic organelles such as chloroplasts and mitochondria.

**12.3** Is the number of potential alleles of a gene directly related to the number of nucleotide pairs in the gene? Is such a relationship more likely to occur in prokaryotes or in eukaryotes? Why?

**ANS:** It depends on how you define alleles. If every variation in nucleotide sequence is considered to be a different allele, even if the gene product and the phenotype of the organism carrying the mutation are unchanged, then the number of alleles will be directly related to gene size. However, if the nucleotide sequence change must produce an altered gene product or phenotype before it is considered a distinct allele, then there will be a positive correlation, but not a direct relationship, between the number of alleles of a gene and its size in nucleotide pairs. The relationship is more likely to occur in prokaryotes where most genes lack introns. In eukaryotic genes, nucleotide sequence changes within introns are usually neutral; that is, they do not affect the activity of the gene product or the phenotype of the organism. Thus, in the case of eukaryotic genes with introns, there may be no correlation between gene size and number of alleles producing altered phenotypes.

**12.4** Why was it necessary to modify Beadle and Tatum's one gene–one enzyme concept of the gene to one gene–one polypeptide?

**ANS:** Several enzymes were shown to contain two or more different polypeptides, and these polypeptides were sometimes controlled by genes that mapped to different chromosomes. Thus, the mutations clearly were not in the same gene.

**12.5** (a) Why is the genetic code a triplet code instead of a singlet or doublet code? (b) How many different amino acids are specified by the genetic code? (c) How many different amino acid sequences are possible in a polypeptide 146 amino acids long?

**ANS:** (a) Singlet and doublet codes provide a maximum of 4 and  $(4)^2$  or 16 codons, respectively. Thus, neither code would be able to specify all 20 amino acids. (b) 20. (c)  $(20)^{146}$ .

**12.6** What types of experimental evidence were used to decipher the genetic code?

**ANS:** Synthetic RNA molecules (polyuridylic acid molecules) containing only the base uracil were prepared. When these synthetic molecules were used to activate *in vitro* protein synthesis systems, small polypeptide containing only the amino acid phenylalanine (polyphenylalanine molecules) was synthesized. Codons composed only of uracil were thus shown to specify phenylalanine. Similar experiments were carried out using synthetic RNA molecules with different base compositions. Later, *in vitro* systems activated with synthetic RNA molecules with known repeating base sequences were developed. Ultimately, *in vitro* systems in which specific aminoacyl-tRNAs were shown to bind to ribosomes activated with

specific mini-mRNAs, which were trinucleotides of known base sequence, were developed and used in codon identification.

- 12.7** In what sense and to what extent is the genetic code (a) degenerate, (b) ordered, and (c) universal?
- ANS:** (a) The genetic code is degenerate in that all but 2 of the 20 amino acids are specified by two or more codons. Some amino acids are specified by six different codons. The degeneracy occurs largely at the third or 3' base of the codons. "Partial degeneracy" occurs where the third base of the codon may be either of the two purines or either of the two pyrimidines and the codon still specifies the same amino acid. "Complete degeneracy" occurs where the third base of the codon may be any one of the four bases and the codon still specifies the same amino acid. (b) The code is ordered in the sense that related codons (codons that differ by a single base change) specify chemically similar amino acids. For example, the codons CUU, AUU, and GUU specify the structurally related amino acids leucine, isoleucine, and valine, respectively. (c) The code appears to be almost completely universal. Known exceptions to universality include strains carrying suppressor mutations that alter the reading of certain codons (with low efficiencies in most cases) and the use of UGA as a tryptophan codon in yeast and human mitochondria.
- 12.8** The thymine analog 5-bromouracil is a chemical mutagen that induces single base-pair substitutions in DNA called transitions (substitutions of one purine for another purine and one pyrimidine for another pyrimidine). Using the known nature of the genetic code (Table 12.1), which of the following amino acid substitutions should you expect to be induced by 5-bromouracil with the highest frequency:
- (a) Met → Val; (b) Met → Leu; (c) Lys → Thr; (d) Lys → Gln; (e) Pro → Arg; or (f) Pro → Gln?
- Why?
- ANS:** (a) Met → Val. This substitution occurs as a result of a transition. All other amino acid substitutions listed would require transversions.
- 12.9** Using the information given in Problem 12.8, would you expect 5-bromouracil to induce a higher frequency of His → Arg or His → Pro substitutions? Why?
- ANS:** Expect a higher frequency of His → Arg substitutions. His → Arg results from a transition; His → Pro would require a transversion (not induced by 5-bromouracil).
- 12.10** What is the minimum number of tRNAs required to recognize the six codons specifying the amino acid leucine?
- ANS:** Because of wobble (Table 12.2), one tRNA can recognize both UUA and UUG codons for leucine. However, it takes two more tRNAs to recognize CUU, CUC, CUA, and CUG. Therefore, a minimum of three tRNAs are required to recognize the six codons for leucine.

**12.11** Characterize ribosomes in general as to size, location, function, and macromolecular composition.

**ANS:** Ribosomes are from 10 to 20 nm in diameter. They are located primarily in the cytoplasm of cells. In bacteria, they are largely free in the cytoplasm. In eukaryotes, many of the ribosomes are attached to the endoplasmic reticulum in the cytoplasm. Ribosomes are complex structures composed of over 50 different polypeptides and three to five different RNA molecules. In both prokaryotes and eukaryotes, ribosomes are the site of translation.

**12.12** (a) Where in the cells of higher organisms do ribosomes originate? (b) Where in the cells are ribosomes most active in protein synthesis?

**ANS:** (a) The nucleus, specifically the nucleoli. (b) The cytoplasm.

**12.13** Identify three different types of RNA that are involved in translation and list the characteristics and functions of each.

**ANS:** Messenger RNA (mRNA) molecules carry genetic information from the chromosomes (where the information is stored) to the ribosomes in the cytoplasm (where the information is expressed during protein synthesis). The linear sequence of triplet codons in an mRNA molecule specifies the linear sequence of amino acids in the polypeptide(s) produced during translation of that mRNA. Transfer RNA (tRNA) molecules are small (about 80 nucleotides long) molecules that carry amino acids to the ribosomes and provide the codon-recognition specificity during translation. Ribosomal RNA (rRNA) molecules provide part of the structure and function of ribosomes; they represent an important part of the machinery required for the synthesis of polypeptides.

**12.14.** (a) How is messenger RNA related to polysome formation? (b) How does rRNA differ from mRNA and tRNA in specificity? (c) How does the tRNA molecule differ from that of DNA and mRNA in size and helical arrangement?

**ANS:** (a) Polysomes are formed when two or more ribosomes are simultaneously translating the same mRNA molecule. Ribosomes are usually spaced about 90 nucleotides apart on an mRNA molecule. Thus, polysome size is determined by mRNA size. (b) A ribosome, which contains rRNA molecules, can participate in the synthesis of any polypeptide specified by the ribosome-associated mRNA. In that sense, rRNA is *nonspecific*. Messenger RNAs and tRNAs, in contrast, are *specific*, in directing the synthesis of a particular polypeptide or set of polypeptides (mRNA) or in attaching to a particular amino acid (tRNA). (c) Transfer RNA molecules are much smaller (about 80 nucleotides) than DNA or mRNA molecules. They are single-stranded molecules but have complex secondary structures because of the base pairing between different segments of the molecules.

**12.15** Outline the process of aminoacyl-tRNA formation.

**ANS:** A specific aminoacyl-tRNA synthetase catalyzes the formation of an amino acid-AMP complex from the appropriate amino acid and ATP (with the release of pyrophosphate). The same enzyme then catalyzes the formation of the aminoacyl-tRNA complex, with the release of AMP. Both the amino acid-AMP and aminoacyl-tRNA linkages are high-energy phosphate bonds.

**12.16** How is translation (a) initiated and (b) terminated?

**ANS:** (a) Translation is initiated by a complex reaction involving mRNA, ribosomes, initiation factors (IF-1, IF-2, and IF-3), GTP, the initiator codon AUG, and a special initiator tRNA ( $tRNA_i^{Met}$ ). It also appears to involve a base-pairing interaction between a base sequence near the 3'-end of the 16S rRNA and a base sequence in the "leader sequence" of the mRNA. (b) Translation is terminated by recognition of one or more of the chain-termination codons (UAG, UAA, and UGA) by the appropriate protein release factor (RF-1 or RF-2).

**12.17** Of what significance is the wobble hypothesis?

**ANS:** Crick's wobble hypothesis explains how the anticodon of a given tRNA can base-pair with two or three different mRNA codons. Crick proposed that the base-pairing between the 5' base of the anticodon in tRNA and the 3' base of the codon in mRNA was less stringent than normal and thus allowed some "wobble" at this site. As a result, a single tRNA often recognizes two or three of the related codons specifying a given amino acid (see Table 12.2).

**12.18** If the average molecular mass of an amino acid in a particular polypeptide is 100 daltons, about how many nucleotides will be present in an mRNA coding sequence specifying this polypeptide, which has a molecular mass of 27,000 daltons?

**ANS:** At least 813 nucleotides [= (270 aa  $\times$  3) + 3 nucleotides for termination codon].

**12.19** The bases A, G, U, C, I (inosine) all occur at the 5' positions of anticodons in tRNAs.

(a) Which base can pair with three different bases at the 3' positions of codons in mRNA? (b) What is the minimum number of tRNAs required to recognize all codons of amino acids specified by codons with complete degeneracy?

**ANS:** (a) Inosine. (b) Two.

**12.20** Assume that in the year 2025, the first expedition of humans to Mars discovers several Martian life forms thriving in hydrothermal vents that exist below the planet's surface. Several teams of molecular biologists extract proteins and nucleic acids from these organisms and make some momentous discoveries. Their first discovery is that the proteins in Martian life forms contain only 14 different amino acids instead of the 20 present in life

forms on Earth. Their second discovery is that the DNA and RNA in these organisms have only two different nucleotides instead of the four nucleotides present in living organisms on Earth. (a) Assuming that transcription and translation work similarly in Martians and Earthlings, what is the minimum number of nucleotides that must be present in the Martian codon to specify all the amino acids in Martians? (b) Assuming that the Martian code proposed above has translational start-and-stop signals, would you expect the Martian genetic code to be degenerate like the genetic code used on Earth?

**ANS:** (a) Two nucleotides in all combinations of four ( $2^4$ ) would produce 16 codons. Therefore, the minimum number of nucleotides comprising the Martian genetic code must be four. (b) Sixteen codons would allow code words for 14 amino acids, one initiation codon, and a translational termination codon. The Martian genetic code could not be degenerate.

**12.21** What are the basic differences between translation in prokaryotes and in eukaryotes?

**ANS:** Translation occurs by very similar mechanisms in prokaryotes and eukaryotes; however, there are some differences. (1) In prokaryotes, the initiation of translation involves base-pairing between a conserved sequence (AGGAGG)—the Shine-Dalgarno box—in mRNA and a complementary sequence near the 3' end of the 16S rRNA. In eukaryotes, the initiation complex forms at the 5' end of the transcript when a cap-binding protein interacts with the 7-methyl guanosine on the mRNA. The complex then scans the mRNA processively and initiates translation (with a few exceptions) at the AUG closest to the 5' terminus. (2) In prokaryotes, the amino group of the initiator methionyl-tRNA $_i^{Met}$  is formylated; in eukaryotes, the amino group of methionyl-tRNA $_i^{Met}$  is not formylated. (3) In prokaryotes, two soluble protein release factors (RFs) are required for chain termination. RF-1 terminates polypeptides in response to UAA and UAG codons; RF-2 terminates chains in response to UAA and UGA codons. In eukaryotes, one release factor responds to all three termination codons.

**12.22** What is the function of each of the following components of the protein-synthesizing apparatus:

(a) Aminoacyl-tRNA synthetase. (b) Release factor 1. (c) Peptidyl transferase. (d) Initiation factors. (e) Elongation factor G

**ANS:** (a) Attachment of an amino acid to the correct tRNA. (b) Recognition of termination codons UAA and UAG and release of the nascent polypeptide from the tRNA in the *P* site of the ribosome. (c) Formation of a peptide bond between the amino group of the aminoacyl-tRNA in the *A* site and the carboxyl group of the growing polypeptide on the tRNA in the *P* site. (d) Formation of the initiation complex required for translation; all steps leading up to peptide bond formation. (e) Translocation of the peptidyl-tRNA from the *A* site on the ribosome to the *P* site.

**12.23** An *E. coli* gene has been isolated and shown to be 68 nm long. What is the maximum number of amino acids that this gene could encode?

**ANS:** Assuming 0.34 nm per nucleotide pair in B-DNA, a gene 68 nm long would contain 200 nucleotide pairs. Given the triplet code, this gene would contain  $200/3 = 66.7$  triplets, one of which must specify chain termination. Disregarding the partial triplet, this gene could encode a maximum of 65 amino acids.

**12.24** (a) What is the difference between a nonsense mutation and a missense mutation? (b) Are nonsense or missense mutations more frequent in living organisms? (c) Why?

**ANS:** (a) A nonsense mutation changes a codon specifying an amino acid to a chain-termination codon, whereas a missense mutation changes a codon specifying one amino acid to a codon specifying a different amino acid. (b) Missense mutations are more frequent. (c) Of the 64 codons, only three specify chain termination. Thus, the number of possible missense mutations is much larger than the number of possible nonsense mutations. Moreover, nonsense mutations almost always produce non-functional gene products. As a result, nonsense mutations in essential genes are usually lethal in the homozygous state.

**12.25** The human  $\alpha$ -globin chain is 141 amino acids long. How many nucleotides in mRNA are required to encode human  $\alpha$ -globin?

**ANS:** 426 nucleotides— $3 \times 141 = 423$  specifying amino acids plus three (one codon) specifying chain termination.

**12.26** What are the functions of the *A*, *P*, and *E* aminoacyl-tRNA binding sites on the ribosome?

**ANS:** The incoming aminoacyl-tRNA enters the *A* site of the ribosome, the nascent polypeptide-tRNA occupies the *P* site, and the uncharged exiting tRNA occupies the *E* site.

**12.27** (a) In what ways does the order in the genetic code minimize mutational lethality? (b) Why do base-pair changes that cause the substitution of a leucine for a valine in the polypeptide gene product seldom produce a mutant phenotype?

**ANS:** (a) Related codons often specify the same or very similar amino acids. As a result, single base-pair substitutions frequently result in the synthesis of identical proteins (degeneracy) or proteins with amino acid substitutions involving very similar amino acids. (b) Leucine and valine have very similar structures and chemical properties; both have nonpolar side groups and fold into essentially the same three-dimensional structures when present in polypeptides. Thus, substitutions of leucine for valine or valine for leucine seldom alter the function of a protein.

**12.28** (a) What is the function of the Shine–Dalgarno sequence in prokaryotic mRNAs? (b) What effect does the deletion of the Shine–Dalgarno sequence from an mRNA have on its translation?

**ANS:** (a) The Shine–Dalgarno sequence is a conserved polypurine tract, consensus AGGAGG, that is located about seven nucleotides upstream from the AUG initiation codon in mRNAs of prokaryotes. It is complementary to, and is believed to base-pair with, a sequence near the 5' terminus of the 16S ribosomal RNA. (b) Prokaryotic mRNAs with the Shine–Dalgarno sequence deleted are either not translated or are translated inefficiently.

**12.29** (a) In what ways are ribosomes and spliceosomes similar? (b) In what ways are they different?

**ANS:** (a) Both ribosomes and spliceosomes play essential roles in gene expression, and both are complex macromolecular structures composed of RNA and protein molecules. (b) Ribosomes are located in the cytoplasm; spliceosomes in the nucleus. Ribosomes are larger and more complex than spliceosomes.

**12.30** The 5' terminus of a human mRNA has the following sequence:

5' -GAAGAGACAAGGTCAUGGCCAUAUGC  
UUGUCCAAUCGUUAGCUGCGCAGGAUC-  
GCCUGGG . . . . . 3'

When this mRNA is translated, what amino acid sequence will be specified by this mRNA sequence?

**ANS:** NH<sub>2</sub>-Met-Ala-Ile-Cys-Leu-Phe-Gln-Ser-Leu-Ala-Ala-Gln-Asp-Arg-Pro-Gly-COOH.

**12.31** A partial (5' subterminal) nucleotide sequence of a prokaryotic mRNA is as follows:

5' - . . . . . AGGAGGCUCGAACAUGUCAUAUGC  
GUUCCAAUCGUUAGCUGCGCAGGACCGUCC  
CGGA . . . . . 3'

When this mRNA is translated, what amino acid sequence will be specified by this portion of the mRNA?

**ANS:** Met-Ser-Ile-Cys-Leu-Phe-Gln-Ser-Leu-Ala-Ala-Gln-Asp-Arg-Pro-Gly

**12.32** The following DNA sequence occurs in the nontemplate strand of a gene in a bacterium (the promoter sequence is located to the left but is not shown):

5' - GAATGTCAGAACTGCCATGCTTCATATGAA-  
TAGACCTCTAG - 3'

(a) What is the ribonucleotide sequence of the mRNA molecule that is transcribed from this piece of DNA? (b) What is the amino acid sequence of the polypeptide encoded by this mRNA? (c) If the nucleotide indicated



by the arrow undergoes a mutation that changes T to A, what will be the resulting amino acid sequence following transcription and translation?

**ANS:** (a) 5'-GAAUGUCAGAACUGCCAUGCUUCAUAUG AAUAGACCUCUAG-3' (b) NH<sub>2</sub>-fMet-Ser-Glu-Leu-Pro-Cys-Phe-Ile-COOH (c) NH<sub>2</sub>-fMet-Ser-Glu-Leu-Pro-Cys-Phe-Ile-Arg-Ile-Asp-Leu-COOH.

**12.33** Alan Garen extensively studied a particular nonsense (chain-termination) mutation in the alkaline phosphatase gene of *E. coli*. This mutation resulted in the termination of the alkaline phosphatase polypeptide chain at a position where the amino acid tryptophan occurred in the wild-type polypeptide. Garen induced revertants (in this case, mutations altering the same codon) of this mutant with chemical mutagens that induced single base-pair substitutions and sequenced the polypeptides in the revertants. Seven different types of revertants were found, each with a different amino acid at the tryptophan position of the wild-type polypeptide (termination position of the mutant polypeptide fragment). The amino acids present at this position in the various revertants included tryptophan, serine, tyrosine, leucine, glutamic acid, glutamine, and lysine. Did the nonsense mutation studied by Garen contain a UAG, a UAA, or a UGA nonsense mutation? Explain the basis of your deduction.

**ANS:** (UAG). This is the only nonsense codon that is related to tryptophan, serine, tyrosine, leucine, glutamic acid, glutamine, and lysine codons by a single base-pair substitution in each case.

**12.34** The following DNA sequence occurs in a bacterium (the promoter sequence is located to the left but is not shown).

↓

5'-CAATCATGGACTGCCATGCTTCATATGAATAGTTGACAT-3'  
3'-GTTAGTACCTGACGGTACGAAGTATACTTATCAACTGTA-5'

(a) What is the ribonucleotide sequence of the mRNA molecule that is transcribed from the template strand of this piece of DNA? Assume that both translational start and termination codons are present.

(b) What is the amino acid sequence of the polypeptide encoded by this mRNA?

(c) If the nucleotide indicated by the arrow undergoes a mutation that causes this C:G base pair to be deleted, what will be the polypeptide encoded by the mutant gene?

**ANS:** (a) 5'-CAAUCAUGGACUGCCAUGCUUCAUAUG AAUAGUUGACAU-3' (b) NH<sub>2</sub>-fMet-Asp-Cys-His-Ala-Ser-Tyr-Glu-COOH (c) NH<sub>2</sub>-fMet-Asp-Cys-Met-Leu-His-Met-Asn-Ser-COOH.

## CHAPTER 13

**13.1** Identify the following point mutations represented in DNA and in RNA as (1) transitions, (2) transversions, or (3) reading frameshifts. (a) A to G; (b) C to T; (c) C to G; (d) T to A; (e) UAU ACC UAU to UAU AAC CUA; (f) UUG CUA AUA to UUG CUG AUA.

**ANS:** (a) Transition, (b) transition, (c) transversion, (d) transversion, (e) frameshift, (f) transition.

**13.2** Of all possible missense mutations that can occur in a segment of DNA encoding the amino acid tryptophan, what is the ratio of transversions to transitions if all single base-pair substitutions occur at the same frequency?

**ANS:** 6:1. UGG transitions: UGA (nonsense), UAG (nonsense), CGG (Arg). UGG transversions: UGC (Cys), UGU (Cys), UCG (Ser), UUG (Leu), AGG (Arg), GGG (Gly).

**13.3** Both lethal and visible mutations are expected to occur in fruit flies that are subjected to irradiation. Outline a method for detecting (a) X-linked lethals and (b) X-linked visible mutations in irradiated *Drosophila*.

**ANS:** (a) *CIB* method, (b) attached X method (see Chapter 6).

**13.4** H. J. Muller used the *CIB* technique to identify many radiation-induced recessive lethal mutations on *Drosophila's* X chromosome, which is now known to contain more than a thousand genes. These mutations could be propagated in stock cultures by keeping them in heterozygous condition with the *CIB* chromosome. Would you expect all these lethal mutations to be alleles of one essential X-linked gene, or to be alleles of different essential X-linked genes? Why couldn't H. J. Muller determine the answer to this question experimentally?

**ANS:** The radiation-induced recessive lethal mutations that Muller recovered in his experiments were likely scattered across the entire X chromosome. Thus, they likely affected different genes. However, Muller could not study this issue because he could not carry out a complementation test to determine if any of the lethal mutations were alleles of the same gene. The reason is that to perform the complementation test, Muller would have had to cross females that carried a particular lethal mutation balanced with the *CIB* chromosome to males that carried a different lethal mutation. Because such males are not viable, the required cross cannot be performed.

**13.5** Published spontaneous mutation rates for humans are generally higher than those for bacteria. Does this indicate that individual genes of humans mutate more frequently than those of bacteria? Explain.

**ANS:** Probably not. A human is larger than a bacterium, with more cells and a longer life span. If mutation frequencies are calculated in terms of cell generations, the rates for human cells and bacterial cells are similar.

**13.6** A precancerous condition (intestinal polyposis) in a particular human family group is caused by a single dominant gene. Among the descendants of one woman who died with cancer of the colon, 10 people have died with the same type of cancer and 6 now have intestinal polyposis. All other branches of the large kindred have been carefully examined, and no cases have been found. Suggest an explanation for the origin of the defective gene.

**ANS:** A dominant mutation presumably occurred in the woman in whom the condition was first known.

**13.7** Juvenile muscular dystrophy in humans depends on an X-linked recessive gene. In an intensive study, 33 cases were found in a population of some 800,000 people. The investigators were confident that they had found all cases that were well enough advanced to be detected at the time the study was made. The symptoms of the disease were expressed only in males. Most of those with the disease died at an early age, and none lived beyond 21 years of age. Usually, only one case was detected in a family, but sometimes two or three cases occurred in the same family. Suggest an explanation for the sporadic occurrence of the disease and the tendency for the gene to persist in the population.

**ANS:** The X-linked gene is carried by mothers, and the disease is expressed in half of their sons. Such a disease is difficult to follow in pedigree studies because of the recessive nature of the gene, the tendency for the expression to skip generations in a family line, and the loss of the males who carry the gene. One explanation for the sporadic occurrence and tendency for the gene to persist is that, by mutation, new defective genes are constantly being added to the load already present in the population.

**13.8** Products resulting from somatic mutations, such as the navel orange and the Delicious apple, have become widespread in citrus groves and apple orchards. However, traits resulting from somatic mutations are seldom maintained in animals. Why?

**ANS:** Plants can be propagated vegetatively, but no such methods are available for widespread use in animals.

**13.9** If a single short-legged sheep should occur in a flock, suggest experiments to determine whether the short legs are the result of a mutation or an environmental effect. If due to a mutation, how can one determine whether the mutation is dominant or recessive?

**ANS:** The sheep with short legs could be mated to unrelated animals with long legs. If the trait is expressed in the first generation, it could be presumed to be inherited and to depend on a dominant gene. On the other hand, if it does not appear in the first generation,  $F_1$  sheep could be

crossed back to the short-legged parent. If the trait is expressed in one-half of the backcross progeny, it is probably inherited as a simple recessive. If two short-legged sheep of different sex could be obtained, they could be mated repeatedly to test the hypothesis of dominance. In the event that the trait is not transmitted to the progeny that result from these matings, it might be considered to be environmental or dependent on some complex genetic mechanism that could not be identified by the simple test used in the experiments.

**13.10** How might enzymes such as DNA polymerase be involved in the mode of action of both mutator and antimutator genes (mutant genes that increase and decrease, respectively, mutation rates)?

**ANS:** Enzymes may discriminate among the different nucleotides that are being incorporated. Mutator enzymes may utilize a higher proportion of incorrect nucleotides, whereas antimutator enzymes may select fewer incorrect bases in DNA replication. In the case of the phage T4 DNA polymerase, the relative efficiencies of polymerization and proofreading by the polymerase's 3' → 5' exonuclease activity play key roles in determining the mutation rate.

**13.11** How could spontaneous mutation rates be optimized by natural selection?

**ANS:** If both mutators and antimutators operate in the same living system, an optimum mutation rate for a particular organism in a given environment may result from natural selection.

**13.12** A mutator gene *Dt* in maize increases the rate at which the gene for colorless aleurone (*a*) mutates to the dominant allele (*A*), which yields colored aleurone. When reciprocal crosses were made (i.e., seed parent *dt/dt, a/a* × *Dt/Dt, a/a* and seed parent *Dt/Dt, a/a* × *dt/dt, a/a*), the cross with *Dt/Dt* seed parents produced three times as many dots per kernel as the reciprocal cross. Explain these results.

**ANS:** *Dt* is a mutator gene that induces somatic mutations in developing kernels.

**13.13** The deficiency *Df(1)w<sup>71</sup>* removes 16 contiguous bands from a region near the left end of the *Drosophila* X chromosome. Females homozygous for this deficiency die. However, females heterozygous for it and a *CIB* chromosome are viable and fertile. If such females are mated to males that carry wild-type X and Y chromosomes, what kinds of progeny will appear and in what proportions?

**ANS:** The cross is *Df(1)w<sup>71</sup>/CIB* females × +/Y (wild-type) males. The genotypes of the daughters are *Df(1)w<sup>71</sup>/+* (phenotypically wild-type) and *CIB/+* (bar-eyed). These two classes of daughters will occur in equal proportions. The sons inherit a Y chromosome and either the *Df(1)w<sup>71</sup>* or *CIB* X chromosomes, both of which act as recessive lethals. Thus, no sons will appear in the progeny.

- 13.14** In *Drosophila*, the Y chromosome  $Y \cdot w^+$  has a small piece of the X chromosome translocated to it; this piece contains the wild-type alleles of all the genes missing in  $Df(1)w^{j1}$  mentioned in Problem 13.13. If males carrying  $Y \cdot w^+$  and a wild-type X chromosome are crossed to  $Df(1)w^{j1}/CIB$  females, what kinds of progeny will appear, and in what proportions? How would your answer change if the wild-type X chromosome in the males carried a radiation-induced recessive lethal mutation located within the region that is missing in  $Df(1)w^{j1}$ ? How could these unusual chromosomes be used to devise a scheme that would allow you to carry out complementation tests between two independently induced recessive lethal mutations that map within this region?

**ANS:** In the cross  $Df(1)w^{j1}/CIB$  females  $\times$   $+/Y \cdot w^+$  (wild-type) males, the daughters will be either  $Df(1)w^{j1}/+$  (phenotypically wild-type) or  $CIB/+$  (bar-eyed); these two types of daughters will appear in equal proportions. The sons from the cross will be either  $Df(1)w^{j1}/Y \cdot w^+$  (viable and phenotypically wild-type because the small piece of the X chromosome translocated to the Y chromosome contains the genes that are missing in the deficiency  $Df(1)w^{j1}$ ) or  $CIB/Y \cdot w^+$ . This latter genotype will be viable if the lethal mutation in the *CIB* chromosome resides in the region defined by the deficiency  $Df(1)w^{j1}$ . In that case, bar-eyed males would appear and be as frequent as wild-type males. If the lethal mutation in the *CIB* chromosome resides outside the region defined by  $Df(1)w^{j1}$ , then no bar-eyed males will appear among the progeny.

If the males in the cross carry a radiation-induced lethal (*ril*) mutation in the region defined by  $Df(1)w^{j1}$ —that is, if the cross is  $Df(1)w^{j1}/CIB$  females  $\times$   $ril/Y \cdot w^+$  males (viable because the small piece of the X chromosome carried by the Y chromosome includes the wild-type allele of *ril*)—there will not be any wild-type daughters (genotype  $Df(1)w^{j1}/ril$ ) among the progeny. However, bar-eyed daughters (genotypically  $CIB/ril$ ) should appear unless the lethal mutation in the *CIB* chromosome is allelic to *ril*.

An induced lethal mutation that lies within the region defined by  $Df(1)w^{j1}$  could be tested for complementation with another such lethal mutation. Let's call the first mutation *lethal-1* and the second mutation *lethal-2*. The required cross for the complementation test is  $CIB/lethal-1$  females  $\times$   $lethal-2/Y \cdot w^+$  males. This cross is possible because the small piece of the X chromosome carried by the  $Y \cdot w^+$  chromosome contains the wild-type allele of *lethal-2*; thus, the  $lethal-2/Y \cdot w^+$  males are viable. Among the progeny, we would look for daughters with the genotype  $lethal-1/lethal-2$ , which, because they lack the *CIB* chromosome, will have normal (non-bar) eyes. If these females appear, we know that *lethal-1* and *lethal-2* complement one another; that is, they are mutations in different genes. If these females do not appear, we can conclude that *lethal-1* and *lethal-2* are alleles of the same gene.

- 13.15** If CTT is a DNA triplet (transcribed strand of DNA) specifying glutamic acid, what DNA and mRNA base triplet alterations could account for valine and lysine in position 6 of the  $\beta$ -globin chain?

**ANS:**

Amino Acid	mRNA	DNA
Glutamic acid	— GAA →	— GAA → ← CTT ← Transcribed strand
Valine	— GUA →	— GTA → ← CAT ← ↓ Mutation
Lysine	— AAA →	— AAA → ← TTT ← ↓ Mutation

- 13.16** The bacteriophage T4 genome contains about 50 percent A:T base pairs and 50 percent G:C base pairs. The base analog 2-aminopurine induces A:T  $\rightarrow$  G:C and G:C  $\rightarrow$  A:T base-pair substitutions by undergoing tautomeric shifts. Hydroxylamine is a mutagenic chemical that reacts specifically with cytosine and induces only G:C  $\rightarrow$  A:T substitutions. If a large number of independent mutations were produced in bacteriophage T4 by treatment with 2-aminopurine, what percentage of these mutations should you expect to be induced to mutate back to the wild-type genotype by treatment with hydroxylamine?

**ANS:** About half of the induced mutations would be expected to mutate back to the wild-type genotype.

- 13.17** Assuming that the  $\beta$ -globin chain and the  $\alpha$ -globin chain shared a common ancestor, what mechanisms might explain the differences that now exist in these two chains? What changes in DNA and mRNA codons would account for the differences that have resulted in unlike amino acids at corresponding positions?

**ANS:** Mutations: transitions, transversions, and frameshifts.

- 13.18** In a given strain of bacteria, all of the cells are usually killed when a specific concentration of streptomycin is present in the medium. Mutations that confer resistance to streptomycin occur. The streptomycin-resistant mutants are of two types: some can live with or without streptomycin; others cannot survive unless this drug is present in the medium. Given a streptomycin-sensitive strain of this species, outline an experimental procedure by which streptomycin-resistant strains of the two types could be established.

**ANS:** Irradiate the nonresistant strain and plate the irradiated organisms on a medium containing streptomycin. Those that survive and produce colonies are resistant. They could then be replicated to a medium without streptomycin. Those that survive would be of the first type; those that can live with streptomycin but not without it would be the second type.

**13.19** One stock of fruit flies was treated with 1000 roentgens (r) of X-rays. The X-ray treatment increased the mutation rate of a particular gene by 2 percent. What percentage increases in the mutation rate of this gene would be expected if this stock of flies was treated with X-ray doses of 1500 r, 2000 r, and 3000 r?

**ANS:** 3%; 4%; 6%.

**13.20** Why does the frequency of chromosome breaks induced by X-rays vary with the total dosage and not with the rate at which it is delivered?

**ANS:** Each quantum of energy from the X-rays that is absorbed in a cell has a certain probability of hitting and breaking a chromosome. Hence, the greater the number of quanta of energy or dosage, the more likely the breaks are to occur. The rate at which this dosage is delivered does not change the probability of each quantum inducing a break.

**13.21** A reactor overheats and produces radioactive tritium ( $H^3$ ), radioactive iodine ( $I^{131}$ ), and radioactive xenon ( $Xn^{133}$ ). Why should we be more concerned about radioactive iodine than the other two radioactive isotopes?

**ANS:** Radioactive iodine is concentrated by living organisms and food chains.

**13.22** One person was in an accident and received 50 roentgens (r) of X-rays at one time. Another person received 5 r in each of 20 treatments. Assuming no intensity effect, what proportionate number of mutations would be expected in each person?

**ANS:** The person receiving a total of 100 r would be expected to have twice as many mutations as the one receiving 50 r.

**13.23** A cross was performed in *Neurospora crassa* between a strain of mating type *A* and genotype  $x^+ m^+ z$  and a strain of mating type *a* and genotype  $x m z^+$ . Genes *x*, *m*, and *z* are closely linked and are present in the order  $x-m-z$  on the chromosome. An ascus produced from this cross contained two copies (“identical twins”) of each of the four products of meiosis. If the genotypes of the four products of meiosis showed that gene conversion had occurred at the *m* locus and that reciprocal recombination had occurred at the *x* and *z* loci, what might the genotypes of the four products look like? In the parentheses below, write the genotypes of the four haploid products of meiosis in an ascus showing gene conversion at the *m* locus and reciprocal recombination of the flanking markers (at the *x* and *z* loci).

**Ascus Spore Pairs**

1-2	3-4	5-6	7-8
( )	( )	( )	( )

**ANS:** ( $x^+ m^+ z$ ) ( $x^+ m^+ z^+$ ) ( $x m^+ z$ ) ( $x m z^+$ ) or equivalent.

**13.24** How does nitrous acid induce mutations? What specific end results might be expected in DNA and mRNA from the treatment of viruses with nitrous acid?

**ANS:** Nitrous acid brings about a substitution of an OH group for an  $NH_2$  group in those bases (A, C, and G) having  $NH_2$  side groups. In so doing, adenine is converted into hypoxanthine, which base-pairs with cytosine, and cytosine is converted into uracil, which base-pairs with adenine. The net effects are  $GC \leftrightarrow AT$  base-pair substitutions (see Figure 13.16).

**13.25** Are mutational changes induced by nitrous acid more likely to be transitions or transversions?

**ANS:** Transitions.

**13.26** You are screening three new pesticides for potential mutagenicity by using the Ames test. Two *his<sup>-</sup>* strains resulting from either a frameshift or a transition mutation were used and produced the following results (number of revertant colonies):

Strain 1	Transition Mutant Control (no chemical)	Transition Mutant + Chemical	Transition Mutant + Chemical + Rat liver Enzymes
	Pesticide #1	21	180
Pesticide #2	18	19	17
Pesticide #3	25	265	270

Strain 2	Frameshift Mutant Control (no chemical)	Frameshift Mutant + Chemical	Frameshift Mutant + Chemical + Rat liver Enzymes
	Pesticide #1	5	4
Pesticide #2	7	5	93
Pesticide #3	6	9	7

What type of mutations, if any, do the three pesticides induce?

**ANS:** P #1—Causes transition mutation. Liver enzymes convert it into nonmutagen. Does not cause frameshift mutations. P #2—Does not cause transition mutations. Liver enzymes convert it into a frameshift mutagen. P #3—Causes transition mutations. Liver enzymes have no effect on mutagenicity. Does not cause frameshift mutations.

**13.27** How does the action and mutagenic effect of 5-bromouracil differ from that of nitrous acid?

**ANS:** Nitrous acid acts as a mutagen on either replicating or nonreplicating DNA and produces transitions from A to G or C to T, whereas 5-bromouracil does not affect nonreplicating DNA but acts during the replication process causing  $GC \leftrightarrow AT$  transitions. 5-Bromouracil must be incorporated into DNA during the replication process in order to induce mispairing of bases and thus mutations.

**13.28** Sydney Brenner and A. O. W. Stretton found that nonsense mutations did not terminate polypeptide synthesis in the *rII* gene of the bacteriophage T4 when these mutations were located within a DNA sequence interval in which a single nucleotide insertion had been made on one end and a single nucleotide deletion had been made on the other. How can this finding be explained?

**ANS:** The reading frame will be shifted between the two frameshift mutations. This shift in reading frame does not read the normal nonsense codon and termination does not occur.

**13.29** Seymour Benzer and Ernst Freese compared spontaneous and 5-bromouracil-induced mutants in the *rII* gene of the bacteriophage T4; the mutagen increased the mutation rate ( $rII^+ \rightarrow rII$ ) several hundred times above the spontaneous mutation rate. Almost all (98 percent) of the 5-bromouracil-induced mutants could be induced to revert to wild-type ( $rII \rightarrow rII^+$ ) by 5-bromouracil treatment, but only 14 percent of the spontaneous mutants could be induced to revert to wild-type by this treatment. Discuss the reason for this result.

**ANS:** 5-BU causes GC  $\leftrightarrow$  AT transitions. 5-BU can, therefore, revert almost all of the mutations that it induces by enhancing the transition event that is the reverse of the one that produced the mutation. In contrast, the spontaneous mutations will include transversions, frameshifts, deletions, and other types of mutations, including transitions. Only the spontaneous transitions will show enhanced reversion after treatment with 5-BU.

**13.30** How do acridine-induced changes in DNA result in inactive proteins?

**ANS:** Mutations induced by acridine dyes are primarily insertions or deletions of single base-pairs. Such mutations alter the reading frame (the in-phase triplets specifying mRNA codons) for that portion of the gene distal (relative to the direction of transcription and translation) to the mutation (see Figure 13.7b). This would be expected to totally change the amino acid sequences of polypeptides distal to the mutation site and produce inactive polypeptides. In addition, such frameshift mutations frequently produce in-frame termination codons that result in truncated proteins.

Use the known codon-amino acid assignments (Table 12.1) to work the following problems.

**13.31** Mutations in the genes encoding the  $\alpha$ - and  $\beta$ -subunits of hemoglobin lead to blood diseases such as thalassemia and sickle-cell anemia. You have found a family in China in which some members suffer from a new genetic form of anemia. The DNA sequences at the 5' end of the non-template strand of the normal and mutant DNA encoding the  $\alpha$  subunit of hemoglobin are as follows:

Normal 5-ACGTTATGCCGTACTGCCAGCTAAC  
TGCTAAAGAACAATTA.....-3

Mutant 5-ACGTTATGCCGTACTGCCAGCTAAC  
CTGCTAAAGAACAATTA....-3

(a) What type of mutation is present in the mutant hemoglobin gene? (b) What are the codons in the translated portion of the mRNA transcribed from the normal and mutant genes? (c) What are the amino acid sequences of the normal and mutant polypeptides?

**ANS:** (a) Frameshift due to the insertion of C at the 9th, 10th, or 11th nucleotide from the 5' end. (b) Normal: 5'-AUGCCGUACUGCCAGCUAACUGCUAAAGAACAUA-3'. Mutant: 5'-AUGCCCGUACUGCCAGCUAACUGCUAAAGAACAUA-3'. (c) Normal: NH<sub>2</sub>-Met-Pro-Tyr-Cys-Gln-Leu-Thr-Ala-Lys-Glu-Gln-Leu. Mutant: NH<sub>2</sub>-Met-Pro-Val-Leu-Pro-Ala-Asn-Cys.

**13.32** Bacteriophage MS2 carries its genetic information in RNA. Its chromosome is analogous to a polygenic molecule of mRNA in organisms that store their genetic information in DNA. The MS2 minichromosome encodes four polypeptides (i.e., it has four genes). One of these four genes encodes the MS2 coat protein, a polypeptide of 129 amino acids long. The entire nucleotide sequence in the RNA of MS2 is known. Codon 112 of the coat protein gene is CUA, which specifies the amino acid leucine. If you were to treat a replicating population of bacteriophage MS2 with the mutagen 5-bromouracil, what amino acid substitutions would you expect to be induced at position 112 of the MS2 coat protein (i.e., Leu  $\rightarrow$  other amino acid)? (Note: Bacteriophage MS2 RNA replicates using a complementary strand of RNA and base-pairing as DNA.)

**ANS:** Proline and serine.

**13.33** Would the different amino acid substitutions induced by 5-bromouracil at position 112 of the coat polypeptide that you indicated in Problem 13.32 be expected to occur with equal frequency? If so, why? If not, why not? Which one(s), if any, would occur more frequently?

**ANS:** No. Leucine  $\rightarrow$  proline would occur more frequently. Leu (CUA)  $\rightarrow$  5-BU  $\rightarrow$  Pro (CCA) occurs by a single base-pair transition, whereas Leu (CUA)  $\rightarrow$  5-BU  $\rightarrow$  Ser (UCA) requires two base-pair transitions. Recall that 5-bromouracil (5-BU) induces only transitions (see Figure 13.15).

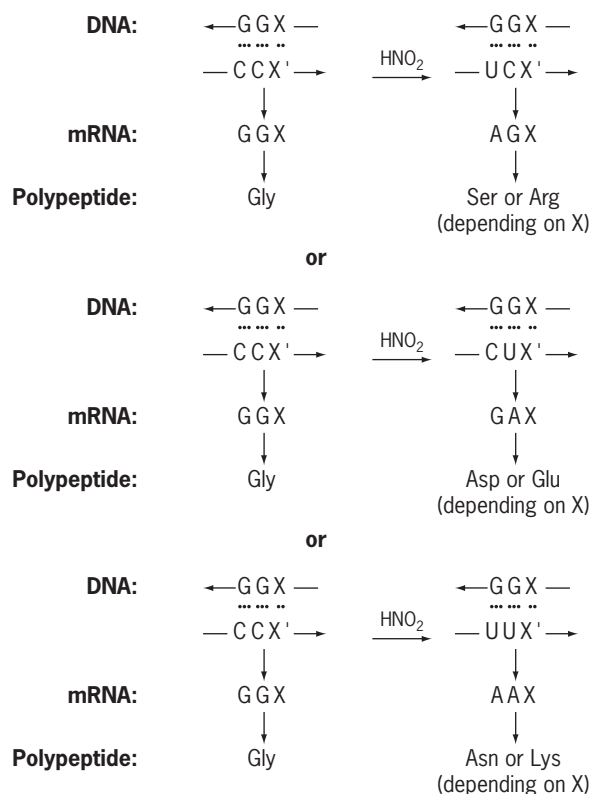
**13.34** Would such mutations occur if a nonreplicating suspension of MS2 phage was treated with 5-bromouracil?

**ANS:** No. 5-Bromouracil is mutagenic only to replicating nucleic acids.

**13.35** Recall that nitrous acid deaminates adenine, cytosine, and guanine (adenine  $\rightarrow$  hypoxanthine, which base-pairs with cytosine; cytosine  $\rightarrow$  uracil, which base-pairs with adenine; and guanine  $\rightarrow$  xanthine, which base-pairs with cytosine). Would you expect nitrous acid to induce any mutations that result in the substitution of another amino acid for a glycine residue in a wild-type polypeptide (i.e., glycine  $\rightarrow$

another amino acid) if the mutagenesis were carried out on a suspension of mature (nonreplicating) T4 bacteriophage? (Note: After the mutagenic treatment of the phage suspension, the nitrous acid is removed. The treated phage is then allowed to infect *E. coli* cells to express any induced mutations.) If so, by what mechanism? If not, why not?

**ANS:** Yes:



*Note:* The X at the third position in each codon in mRNA and in each triplet of base pairs in DNA refers to the fact that there is complete degeneracy at the third base in the glycine codon. Any base may be present in the codon, and it will still specify glycine.

**13.36** Keeping in mind the known nature of the genetic code, the information given about phage MS2 in Problem 13.32, and the information you have learned about nitrous acid in Problem 13.35, would you expect nitrous acid to induce any mutations that would result in amino acid substitutions of the type glycine → another amino acid if the mutagenesis were carried out on a suspension of mature (nonreplicating) MS2 bacteriophage? If so, by what mechanism? If not, why not?

**ANS:** No. The glycine codon is GGX, where X can be any one of the four bases. Because of this complete degeneracy at the third position of the glycine codon, changing X to any other base will have no effect (i.e., the codon will still specify glycine). Nitrous acid deaminates guanine (G) to xanthine, but xanthine still base-pairs with cytosine. Thus, guanine is not a target for mutagenesis by nitrous acid.

**13.37** Would you expect nitrous acid to induce a higher frequency of Tyr → Ser or Tyr → Cys substitutions? Why?

**ANS:** Tyr → Cys substitutions; Tyr to Cys requires a transition, which is induced by nitrous acid. Tyr to Ser would require a transversion, and nitrous acid is not expected to induce transversions.

**13.38** Which of the following amino acid substitutions should you expect to be induced by 5-bromouracil with the highest frequency? (a) Met → Leu; (b) Met → Thr; (c) Lys → Thr; (d) Lys → Gln; (e) Pro → Arg; or (f) Pro → Gln? Why?

**ANS:** (b) Met → Thr. 5-Bromouracil induces transitions, not transversions. All other changes listed require transversions.

**13.39** The wild-type sequence of part of a protein is NH<sub>2</sub>-Trp-Trp-Trp-Met-Arg-Glu-Trp-Thr-Met

Each mutant in the following table differs from wild-type by a single point mutation. Using this information, determine the mRNA sequence coding for the wild-type polypeptide. If there is more than one possible nucleotide, list all possibilities.

Mutant	Amino Acid Sequence of Polypeptide
1	Trp-Trp-Trp Met
2	Trp-Trp-Trp-Met-Arg-Asp-Trp-Thr-Met
3	Trp-Trp-Trp-Met-Arg-Lys-Trp-Thr-Met
4	Trp-Trp-Trp-Met-Arg-Glu-Trp-Met-Met

**ANS:** 5'-UGG-UGG-UGG-AUG-CGA(or AGA)-GAA(or GAG)-UGG-AUG-3'

**13.40** Acridine dyes such as proflavin are known to induce primarily single base-pair additions and deletions. Suppose that the wild-type nucleotide sequence in the mRNA produced from a gene is

5'-AUGCCCUUUGGGAAAGGGUUUCCCUAA-3'

Also, assume that a mutation is induced within this gene by proflavin, and, subsequently, a revertant of this mutation is similarly induced with proflavin and shown to result from a second-site suppressor mutation within the same gene. If the amino acid sequence of the polypeptide encoded by this gene in the revertant (double mutant) strain is

NH<sub>2</sub>-Met-Pro-Phe-Gly-Glu-Arg-Phe-Pro-COOH

what would be the most likely nucleotide sequence in the mRNA of this gene in the revertant (double mutant)?

**ANS:** 5'-AUGCCCUUUGGGAAAGGGUUUCCCUAA-3'

**13.41** Eight independently isolated mutants of *E. coli*, all of which are unable to grow in the absence of histidine (his<sup>-</sup>), were examined in all possible *cis* and *trans* heterozygotes (partial diploids). All of the *cis* heterozygotes were able to grow in the absence of histidine. The *trans* heterozygotes yielded two different responses: some of them grew in the absence of histidine; others did not. The experimental results, using "+" to indicate growth and "0" to indicate no growth, are given in the accompanying table. How many genes are defined by these eight mutations? Which mutant strains carry mutations in the same gene(s)?

**Growth of *Trans* Heterozygotes (without Histidine)**

Mutant	1	2	3	4	5	6	7	8
8	0	0	0	0	0	0	1	0
7	+	+	+	+	+	+	0	
6	0	0	0	0	0	0		
5	0	0	0	0	0			
4	0	0	0	0				
3	0	0	0					
2	0	0						
1	0							

**ANS:** Two genes; mutations 1, 2, 3, 4, 5, 6, and 8 are in one gene; mutation 7 is in a second gene.

**13.42** Assume that the mutants described in Problem 13.41 yielded the following results. How many genes would they have defined? Which mutations would have been in the same gene(s)?

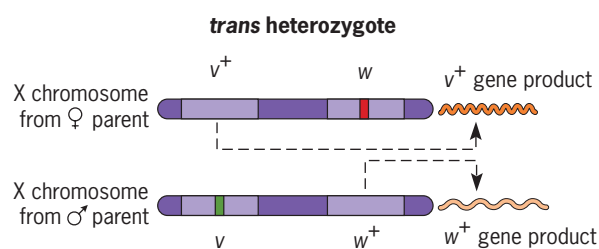
Mutant	1	2	3	4	5	6	7	8
8	+	+	+	+	+	+	0	0
7	+	+	+	+	+	+	0	
6	+	+	+	+	0	0		
5	+	+	+	+	0			
4	+	+	0	0				
3	+	+	0					
2	0	0						
1	0							

**ANS:** Four genes; mutations 1 and 2 in one gene; mutations 3 and 4 in a second gene; mutations 5 and 6 in a third gene; and mutations 7 and 8 in a fourth gene.

**13.43** In *Drosophila*, *white*, *white cherry*, and *vermillion* are all sex-linked mutations affecting eye color. All three mutations are recessive to their wild-type allele(s) for red eyes. A white-eyed female crossed with a vermillion-eyed male produces white-eyed male offspring and red-eyed (wild-type) female offspring. A white-eyed female crossed with a white cherry-eyed male produces white-eyed sons and light cherry-eyed daughters. Do these results indicate whether or not any of the three mutations affecting eye color are located in the same gene? If so, which mutations?

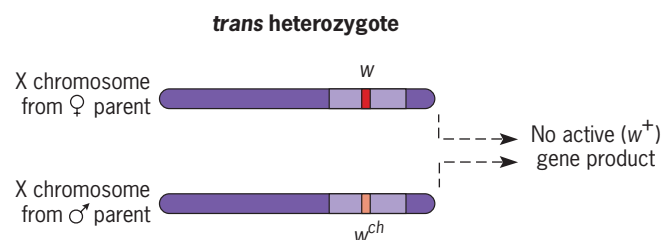
**ANS:** The complementation test for allelism involves placing mutations pairwise in a common protoplasm in the *trans* configuration and determining whether the resulting *trans* heterozygotes have wild-type or mutant phenotypes. If the two mutations are in different genes, the two mutations will complement each other, because the wild-type copies of each gene will produce functional

gene products (see Figure 13.21a). However, if the two mutations are in the same gene, both copies of the gene in the *trans* heterozygote will produce defective gene products, resulting in a mutant phenotype (see Figure 13.21b). When complementation occurs, the *trans* heterozygote will have the wild-type phenotype. Thus, the complementation test allows one to determine whether any two recessive mutations are located in the same gene or in different genes. Because the mutations of interest are sex-linked, all the male progeny will have the same phenotype as the female parent. They are hemizygous, with one X chromosome obtained from their mother. In contrast, the female progeny are *trans* heterozygotes. In the cross between the white-eyed female and the vermillion-eyed male, the female progeny have red eyes, the wild-type phenotype. Thus, the *white* and *vermillion* mutations are in different genes, as illustrated in the following diagram:



Complementation yields wild-type phenotype; both  $v^+$  and  $w^+$  gene products are produced in the *trans* heterozygote.

In the cross between a white-eyed female and a white cherry-eyed male, the female progeny have light cherry-colored eyes (a mutant phenotype), not wild-type red eyes as in the first cross. Since the *trans* heterozygote has a mutant phenotype, the two mutations, *white* and *white cherry*, are in the same gene:



No  $w^+$  gene product; therefore, mutant phenotype.

**13.44** The *loz* (*lethal on Z*) mutants of bacteriophage X are conditional lethal mutants that can grow on *E. coli* strain Y but cannot grow on *E. coli* strain Z. The results shown in the following table were obtained when seven *loz* mutants were analyzed for complementation by infecting *E. coli* strain Z with each possible pair of mutants. A “+” indicates that progeny phage were produced in the infected cells, and a “0” indicates that no progeny phage were produced. All possible *cis* tests were also done, and all *cis* heterozygotes produced wild-type yields of progeny phage.

Mutant	1	2	3	4	5	6	7
7	+	+	0	+	0	0	0
6	+	+	+	+	+	0	
5	+	+	0	+	0		
4	0	0	+	0			
3	+	+	0				
2	0	0					
1	0						

Propose three plausible explanations for the apparently anomalous complementation behavior of *loz* mutant number 7. (b) What simple genetic experiments can be used to distinguish between the three possible explanations? (c) Explain why specific outcomes of the proposed experiments will distinguish between the three possible explanations.

**ANS:** Mutations 1, 2, and 4 do not complement one another and thus appear to be located in the same gene, as do mutations 3 and 5. The anomaly is that mutation 7 does not complement mutations 3, 5, and 6, even though mutation 6 does complement mutations 3 and 5. (a) There are three simple explanations of the seemingly anomalous complementation behavior of mutation 7. (1) It is a deletion spanning all or parts of two genes. (2) It is a double mutation with defects in two genes. (3) It is a polar mutation—a nonsense mutation that interferes with the expression of downstream genes—in the promoter-proximal gene of a multigenic transcription unit. (b) Three simple genetic operations will distinguish between these three possibilities. (1) Reversion. Plate a large number of mutant 7 phage on *E. coli* strain Z and look for wild-type revertants. (2) Backcross mutant 7 to wild-type phage and test the mutant progeny for the ability to complement mutations 3 and 6. (3) Introduce F's carrying tRNA nonsense suppressor genes into *E. coli* strain Z and determine whether any of them suppress the *loz7* mutation. (c) If mutation 7 is a deletion, it will not revert, and, if it is a double mutation, the reversion rate will probably be below the level of detection in your experiment. On the other hand, if it is a polar nonsense mutation, *loz*<sup>+</sup> revertants will be obtained. If mutation 7 is a deletion, no new genotypes will be produced in the backcross to wild-type. However, if it is a double mutation, some recombinant single-mutant progeny will be produced in the backcross to wild-type, and these single mutations will complement either mutation 3 or mutation 6. If mutation 7 is a polar nonsense mutation, it should be suppressed by one or more of the tRNA suppressor genes introduced into *E. coli* strain Z.

## CHAPTER 14

**14.1** (a) In what ways is the introduction of recombinant DNA molecules into host cells similar to mutation? (b) In what ways is it different?

**ANS:** (a) Both introduce new genetic variability into the cell. In both cases, only one gene or a small segment of DNA representing a small fraction of the total genome is changed or added to the genome. The vast majority of the genes of the organism remain the same. (b) The introduction of recombinant DNA molecules, if they come from a very different species, is more likely to result in a novel, functional gene product in the cell, if the introduced gene (or genes) is capable of being expressed in the foreign protoplasm. The introduction of recombinant DNA molecules is more analogous to duplication mutations (see Chapter 6) than to other types of mutations.

**14.2** Listed in this question are four different single strands of DNA. Which of these, in their double-stranded form, would you expect to be cleaved by a restriction endonuclease?

- (a) ACTCCAGAATTCACTCCG
- (b) GCCTCATTCGAAGCCTGA
- (c) CTCGCCAATTGACTCGTC
- (d) ACTCCACTCCCGACTCCA

**ANS:** Restriction endonucleases cleave at palindromes in double-stranded DNA. A palindrome (indicated in bold-faced letters) can be found in all the double-stranded sequences except d. Therefore, sequence d would not be cleaved by a restriction endonuclease, whereas a, b, and c could be cleaved by the appropriate enzyme.

- (a) ACTCCAGA**AATTC**ACTCCG  
TGCGGT**CTTAA**GTGAGGC
- (b) GCCTCAT**TCGA**AGCCTGA  
CGGAG**TAA**GCTTCGGACT
- (c) CTCGCC**AATTG**ACTCGTC  
GAGCG**GTTAA**CTGAGCAG
- (d) ACTCCACTCCCGACTCCA  
TGAGGAGAGGGCTGAGGT

**14.3** If the sequence of base pairs along a DNA molecule occurs strictly at random, what is the expected frequency of a specific restriction enzyme recognition sequence of length (a) four and (b) six base pairs?

**ANS:** (a)  $(1/4)^4 = 1/256$ ; (b)  $(1/4)^6 = 1/4096$

**14.4** In what ways do restriction endonucleases differ from other endonucleases?

**ANS:** Restriction endonucleases recognize and cut specific nucleotide sequences in DNA. Most other endonucleases are not sequence-specific; many cut DNA sequences at random.



**14.5** Of what value are recombinant DNA and gene-cloning technologies to geneticists?

**ANS:** Recombinant DNA and gene-cloning techniques allow geneticists to isolate essentially any gene or DNA sequence of interest and to characterize it structurally and functionally. Large quantities of a given gene can be obtained in pure form, which permits one to determine its nucleotide-pair sequence (to “sequence it” in common lab jargon). From the nucleotide sequence and our knowledge of the genetic code, geneticists can predict the amino acid sequence of any polypeptide encoded by the gene. By using an appropriate subclone of the gene as a hybridization probe in northern blot analyses, geneticists can identify the tissues in which the gene is expressed. Based on the predicted amino acid sequence of a polypeptide encoded by a gene, geneticists can synthesize oligopeptides and use these to raise antibodies that, in turn, can be used to identify the actual product of the gene and localize it within cells or tissues of the organism. Thus, recombinant DNA and gene-cloning technologies provide very powerful tools with which to study the genetic control of essentially all biological processes. These tools have played major roles in the explosive progress in the field of biology during the last three decades.

**14.6** What determines the sites at which DNA molecules will be cleaved by a restriction endonuclease?

**ANS:** The nucleotide-pair sequence. Restriction endonucleases recognize a specific nucleotide-pair sequence in DNA regardless of the source of the DNA. In most cases, this is a 4 or 6 nucleotide-pair sequence; in a few cases, the recognition sequence is longer (e.g., 8 nucleotide pairs). Most restriction enzymes cleave the two strands of the DNA at a specific position (between the same two adjacent nucleotides in each strand) within the recognition sequence. A few restriction enzymes bind at a specific recognition sequence but cut the DNA at a nearby site outside of the recognition sequences. Some restriction endonucleases cut both strands between the same two nucleotide pairs (“blunt end” cutters), whereas others cut the two strands at different positions and yield complementary single-stranded ends (“sticky or staggered end” cutters). See Table 14.1 for examples.

**14.7** Restriction endonucleases are invaluable tools for biologists. However, genes encoding restriction enzymes obviously did not evolve to provide tools for scientists. Of what possible value are restriction endonucleases to the microorganisms that produce them?

**ANS:** Restriction endonucleases are believed to provide a kind of primitive immune system to the microorganisms that produce them—protecting their genetic material from “invasion” by foreign DNAs from viruses or other pathogens or just DNA in the environment that might be taken up by the microorganism. Obviously, these

microorganisms do not have a sophisticated immune system like that of higher animals (see Chapter 22).

**14.8** Why is the DNA of a microorganism not degraded by a restriction endonuclease that it produces, even though its DNA contains recognition sequences normally cleaved by the endonuclease?

**ANS:** Microorganisms that produce restriction endonucleases also produce enzymes that modify one or more bases in the recognition sequence for that endonuclease so that it can no longer cleave the DNA at that site. In most cases, the modifying enzyme is a methylase that attaches a methyl group to one or more of the bases in the recognition sequence. For example, *E. coli* strains that produce the restriction endonuclease *EcoRI* also produce *EcoRI* methylase, an enzyme that transfers a methyl group from *S*-adenosylmethionine to the second (most 3') adenine residue in each strand of the recognition sequence (5-GAATCC-3) producing N<sup>6</sup>-methyladenines at these positions. *EcoRI* cannot cleave DNA that contains N<sup>6</sup>-methyladenine at these positions even if the *EcoRI* recognition sequence is present in this DNA (see Figure 14.1). Thus, if one wishes to digest DNA with *EcoRI*, that DNA must not be isolated from an *E. coli* strain that is producing *EcoRI* methylase.

**14.9** One of the procedures for cloning foreign DNA segments takes advantage of restriction endonucleases such as *HindIII* (see Table 14.1) that produce complementary single-stranded ends. These enzymes produce identical complementary ends on cleaved foreign DNAs and on the vector DNAs into which the foreign DNAs are inserted. Assume that you have inserted your favorite gene into the *HindIII* site in the polycloning region of the Bluescript cloning vector with DNA ligase, have amplified the plasmid containing your gene in *E. coli*, and have isolated a large quantity of gene/Bluescript DNA. How could you excise your favorite gene from the Bluescript vector?

**ANS:** A foreign DNA cloned using an enzyme that produces single-stranded complementary ends can always be excised from the cloning vector by cleavage with the same restriction enzyme that was originally used to clone it. For example, the *HindIII* fragment carrying your favorite can be excised from the Bluescript DNA by cleavage with restriction endonuclease *HindIII*. The human *HindIII* fragment will be flanked in the recombinant plasmid DNA clone by *HindIII* cleavage sites.

**14.10** You are working as part of a research team studying the structure and function of a particular gene. Your job is to clone the gene. A restriction map is available for the region of the chromosome in which the gene is located; the map is as follows:



Your first task is to prepare a genomic DNA library that contains clones carrying the entire gene. Describe how you would prepare such a library in plasmid vector pBluescript II (see Figure 14.3), indicating which restriction enzymes, media, and host cells you would use.

**ANS:** Step 1: Digest genomic DNA isolated from your research organism with *EcoRI*. Step 2: Treat pBluescript II DNA with *EcoRI*. Step 3: Mix the *EcoRI*-digested genomic and vector DNAs under annealing conditions and incubate with DNA ligase. Step 4: Transform *amp<sup>r</sup>* *E. coli* cells carrying the *lacZM15* gene with the resulting ligation products. Step 5: Plate the transformed cells on nutrient agar medium containing Xgal and ampicillin. Only transformed cells will produce colonies in the presence of ampicillin. Step 6: Prepare your genomic DNA library by using bacteria from white colonies; these bacteria will contain pBluescript II DNA with genomic DNA inserts. Bacteria harboring Bluescript II plasmids with no insert will produce blue colonies (see Figure 14.4).

**14.11** Compare the nucleotide-pair sequences of genomic DNA clones and cDNA clones of specific genes of higher plants and animals. What is the most frequent difference that you would observe?

**ANS:** Most genes of higher plants and animals contain non-coding intron sequences. These intron sequences will be present in genomic clones, but not in cDNA clones, because cDNAs are synthesized using mRNA templates and intron sequences are removed during the processing of the primary transcripts to produce mature mRNAs.

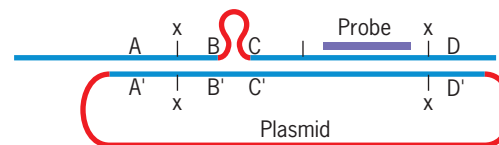
**14.12** Most of the genes of plants and animals that were cloned soon after the development of recombinant DNA technologies were genes encoding products that are synthesized in large quantities in specialized cells. For example, about 90 percent of the protein synthesized in mature red blood cells of mammals consists of  $\alpha$ - and  $\beta$ -globin chains, and the globin genes were among the first mammalian genes cloned. Why were genes of this type so prevalent among the first eukaryotic genes that were cloned?

**ANS:** Higher eukaryotes have very large genomes; for example, the genomes of mammals contain approximately  $3 \times 10^9$  nucleotide pairs. Thus, trying to identify a particular single-copy gene from a clone library is like looking for the proverbial “needle-in-a-haystack.” To accomplish this, one needs a nucleic acid hybridization probe specific for the gene or an antibody probe specific for the gene product. Given a specific cell or tissue type producing the mRNA and/or the protein gene product in large amounts, it was relatively easy to obtain pure mRNA or pure protein to use in making a hybridization or antibody probe, respectively, with which to screen a library for the gene or cDNA of interest. These approaches are much more difficult for the majority of the genes that encode products that represent only a small proportion of the total gene products in any given cell type.

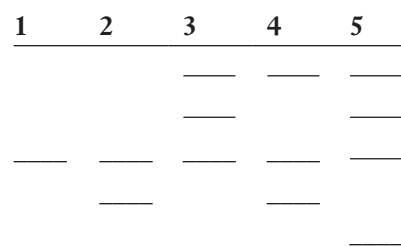
**14.13** Genomic clones of the chloroplastic glutamine synthetase gene (*gln2*) of maize are cleaved into two fragments by digestion with restriction endonuclease *HindIII*, whereas full-length maize *gln2* cDNA clones are not cut by *HindIII*. Explain these results.

**ANS:** The maize *gln2* gene contains many introns, and one of the introns contains a *HindIII* cleavage site. The intron sequences (and thus the *HindIII* cleavage site) are not present in mRNA sequences and thus are also not present in full-length *gln2* cDNA clones.

**14.14** In the following illustration, the upper line shows a gene composed of segments A–D. The lower circle shows a mutant version of this gene, consisting of two fused pieces (A-B, C-D), carried on a plasmid. You attempt a targeted mutagenesis of a diploid cell by transforming cells with the cloned mutant gene. The following diagram shows the desired pairing of the plasmid and chromosome just prior to recombination.



You prepare DNA from the cells, digest it with an enzyme that cuts at x, and hybridize the cleaved DNA with the probe shown above. The following diagram shows a Southern blot of possible results.



(a) Which lane shows fragments produced from DNA in the cell before transformation? (b) Which lane shows fragments produced from DNA in the cell in which the anticipated targeted mutagenesis occurred?

(c) Which of these blot patterns might be expected if two crossovers occurred, one between A and B, and the other between C and D?

**ANS:** (a) 1 (b) 2 (c) 2

**14.15** (a) What experimental procedure is carried out in Southern, northern, and western blot analyses? (b) What is the major difference between Southern, northern, and western blot analyses?

**ANS:** (a) Southern, northern, and western blot procedures all share one common step, namely, the transfer of macromolecules (DNAs, RNAs, and proteins, respectively) that have been separated by gel electrophoresis to a solid support—usually a nitrocellulose or nylon

membrane—for further analysis. (b) The major difference between these techniques is the class of macromolecules that are separated during the electrophoresis step: DNA for Southern blots, RNA for northern blots, and protein for western blots.

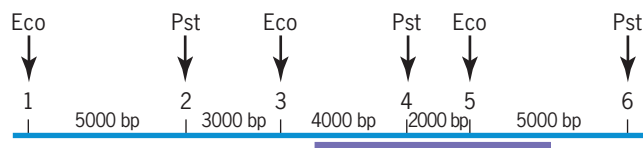
- 14.16** What major advantage does the polymerase chain reaction (PCR) have over other methods for analyzing nucleic acid structure and function?

**ANS:** The PCR technique has much greater sensitivity than any other method available for analyzing nucleic acids. Thus, PCR procedures permit analysis of nucleic acid structure given extremely minute amounts of starting material. DNA sequences can be amplified and structurally analyzed from very small amounts of tissue like blood or sperm in assault and rape cases. In addition, PCR methods permit investigators to detect the presence of rare gene transcripts (e.g., in specific types of cells) that could not be detected by less sensitive procedures such as northern blot analyses or *in situ* hybridization studies.

- 14.17** The cloning vectors in use today contain an origin of replication, a selectable marker gene (usually an antibiotic-resistance gene), and one additional component. What is this component, and what is its function?

**ANS:** All modern cloning vectors contain a “polycloning site” or “multiple cloning site” (MCS)—a cluster of unique cleavage sites for a number of different restriction endonucleases in a nonessential region of the vector into which the foreign DNA can be inserted. In general, the greater the complexity of the MCS—that is, the more restriction endonuclease cleavage sites that are present—the greater the utility of the vector for cloning a wide variety of different restriction fragments. For example, see the MCS present in plasmid Bluescript II shown in Figure 14.3.

- 14.18** The drawing in this problem shows a restriction map of a segment of a DNA molecule. *Eco* refers to locations where the restriction endonuclease *Eco*RI cuts the DNA, and *Pst* refers to locations where the restriction enzyme *Pst*I cuts the DNA. Potential restriction sites are numbered 1–6. Distances between restriction sites are shown on the bottom scale in base pairs (bp). The thick line represents the part of the molecule that has homology with a probe.



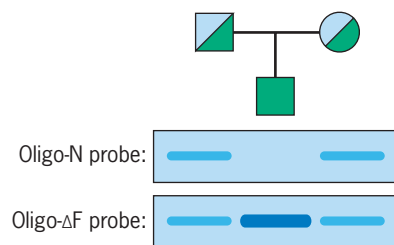
- (a) Assume that individual 1 has restriction sites 1 through 6. If this individual’s DNA is digested with *Pst*I, what are the expected sizes of the DNA fragments that will hybridize with the probe? (b) Assume that individual 2 has a mutation that eliminates site 4. If this individual’s

DNA is digested with *Pst*I, what are the expected sizes of the DNA fragments that will hybridize with the probe? (c) Assume that individual 3 has a mutation that eliminates site 5. If the DNA of this individual is digested with *Pst*I, what are the expected sizes of the DNA fragments that will hybridize with the probe? (d) If the DNA of individual 1 is digested with both *Pst*I and *Eco*RI, what are the expected sizes of the DNA fragments that will hybridize with the probe? (e) If the DNA of individual 3 is digested with both *Pst*I and *Eco*RI, what are the expected sizes of the DNA fragments that will hybridize with the probe?

**ANS:** (a) 7000 bp + 7000 bp; (b) 14,000 bp; (c) 7000 bp + 7000 bp; (d) 4000 bp + 2000 bp + 5000 bp; (e) 4000 bp + 7000 bp.

- 14.19** The cystic fibrosis (*CF*) gene (location: chromosome 7, region q31) has been cloned and sequenced, and studies of *CF* patients have shown that about 70 percent of them are homozygous for a mutant *CF* allele that has a specific three-nucleotide-pair deletion (equivalent to one codon). This deletion results in the loss of a phenylalanine residue at position 508 in the predicted *CF* gene product. Assume that you are a genetic counselor responsible for advising families with *CF* in their pedigrees regarding the risk of *CF* among their offspring. How might you screen putative *CF* patients and their parents and relatives for the presence of the *CF* $\Delta$ 508 mutant gene? What would the detection of this mutant gene in a family allow you to say about the chances that *CF* will occur again in the family?

**ANS:** Because the nucleotide-pair sequences of both the normal *CF* gene and the *CF* $\Delta$ 508 mutant gene are known, labeled oligonucleotides can be synthesized and used as hybridization probes to detect the presence of each allele (normal and  $\Delta$ 508). Under high-stringency hybridization conditions, each probe will hybridize only with the *CF* allele that exhibits perfect complementarity to itself. Since the sequences of the *CF* gene flanking the  $\Delta$ 508 site are known, oligonucleotide PCR primers can be synthesized and used to amplify this segment of the DNA obtained from small tissue explants of putative *CF* patients and their relatives by PCR. The amplified DNAs can then be separated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized to the respective labeled oligonucleotide probes, and the presence of each *CF* allele can be detected by autoradiography. For a demonstration of the utility of this procedure, see Focus on Detection of a Mutant Gene Causing Cystic Fibrosis. In the procedure described there, two synthetic oligonucleotide probes—oligo-N = 3′-CTTTTATAGTAGAAACCAC-5′ and oligo- $\Delta$ F = 3′-TTCTTTTATAGTA—ACCACAA-5′ (the dash indicates the deleted nucleotides in the *CF* $\Delta$ 508 mutant allele) were used to analyze the DNA of *CF* patients and their parents. For confirmed *CF* families, the results of these Southern blot hybridizations with the oligo-N (normal) and oligo-DF (*CF* $\Delta$ 508) labeled probes were often as follows:



Both parents were heterozygous for the normal *CF* allele and the mutant *CF* $\Delta 508$  allele as would be expected for a rare recessive trait, and the CF patient was homozygous for the *CF* $\Delta 508$  allele. In such families, one-fourth of the children would be expected to be homozygous for the  $\Delta 508$  mutant allele and exhibit the symptoms of CF, whereas three-fourths would be normal (not have CF). However, two-thirds of these normal children would be expected to be heterozygous and transmit the allele to their children. Only one-fourth of the children of this family would be homozygous for the normal *CF* allele and have no chance of transmitting the mutant *CF* gene to their offspring. Note that the screening procedure described here can be used to determine which of the normal children are carriers of the *CF* $\Delta 508$  allele: that is, the mutant gene can be detected in heterozygotes as well as in homozygotes.

- 14.20** Cereal grains are major food sources for humans and other animals in many regions of the world. However, most cereal grains contain inadequate supplies of certain of the amino acids that are essential for monogastric animals such as humans. For example, corn contains insufficient amounts of lysine, tryptophan, and threonine. Thus, a major goal of plant geneticists is to produce corn varieties with increased kernel lysine content. As a prerequisite to the engineering of high-lysine corn, molecular biologists need more basic information about the regulation of the biosynthesis and the activity of the enzymes involved in the synthesis of lysine. The first step in the anabolic pathway unique to the biosynthesis of lysine is catalyzed by the enzyme dihydrodipicolinate synthase. Assume that you have recently been hired by a major U.S. plant research institute and that you have been asked to isolate a clone of the nucleic acid sequence encoding dihydrodipicolinate synthase in maize. Briefly describe four different approaches you might take in attempting to isolate such a clone and include at least one genetic approach.

**ANS:** You could attempt to isolate either a dihydrodipicolinate synthase (DHPS) cDNA clone or a DHPS genomic clone. Once you have isolated either DHPS clone, it can be used as a hybridization probe to isolate the other (genomic or cDNA) by screening an appropriate library by *in situ* colony hybridization. Four approaches that have proven effective in isolating other eukaryotic coding sequences of interest are the following: (1) You could obtain a clone of the DHPS gene of a lower eukaryote (a clone of the DHPS gene of *Saccharomyces cerevisiae* is available) or even a prokaryote and use it as a heterologous hybridization probe to screen a maize cDNA

library using low stringency conditions. Sometimes this approach is successful; sometimes it is not successful. Whether or not this approach works depends on how similar the coding sequences of the specific gene of interest are in the two species. (2) You could purify the DHPS enzyme from corn and use the purified protein to produce an antibody to DHPS. This DHPS-specific antibody could then be used to screen a maize cDNA expression library by a protocol analogous to the western blot procedure. (An expression library contains the cDNA coding sequences fused to appropriate transcription and translation signals so that they are expressed in *E. coli* or other host cells in which the cDNA library is prepared.) (3) You could purify the DHPS enzyme from maize and determine the amino acid sequence of its NH<sub>2</sub>-terminus by microsequencing techniques. From the amino acid sequence and the known genetic code, you could predict the possible nucleotide sequences encoding this segment of the protein. Because of the degeneracy in the code, there would be a set of nucleotide sequences that would all specify the same amino acid sequence, and you would not know which one was present in the maize DHPS gene. However, the synthesis of oligonucleotides is now routine and quite inexpensive. Thus, you could synthesize a mixture of oligonucleotides containing all possible coding sequences and use this mixture as a set of hybridization probes to screen an appropriate library by *in situ* colony hybridization. (4) Finally, you might try a simple and very quick genetic approach based on the ability of cDNAs in an expression library to rescue DHPS mutants of *E. coli* or other species that can be transformed at high frequencies. You would obtain a DHPS-deficient mutant of *E. coli* (available from the *E. coli* Genetics Stock Center at Yale University), transform it with your cDNA expression library, and plate the transformed cells on medium lacking diaminopimelic acid (the product of DHPS). DHPS-deficient *E. coli* mutants cannot grow in the absence of diaminopimelic acid; thus, any colonies that grow on your selection plates should be the result of rescue of the DHPS mutant bacteria by corn DHPS encoded by cDNAs in the library. This entire screening procedure can be carried out in 3 or 4 days; thus, it is much simpler than the preceding approaches. In fact, David A. Frisch first isolated a maize DHPS cDNA by this simple, but powerful genetic approach. However, that this approach would only be expected to work in the case of enzymes that are active as monomers or homomultimers; it is not applicable when the active form of the enzyme is a heteromultimer.

- 14.21** You have just isolated a mutant of the bacterium *Shigella dysenteriae* that is resistant to the antibiotic kanamycin, and you want to characterize the gene responsible for this resistance. Design a protocol using genetic selection to identify the gene of interest.

**ANS:** Genetic selection is the most efficient approach to cloning genes of this type. Prepare a genomic library in an expression vector such as Bluescript (see Figure 14.3)

using DNA from the kanamycin-resistant strain of *Shigella dysenteriae*. Then, screen the library for the kanamycin-resistance gene by transforming kanamycin-sensitive *E. coli* cells with the clones in the library and plating the transformed cells on medium containing kanamycin. Only cells that are transformed with the kanamycin-resistance gene will produce colonies in the presence of kanamycin.

- 14.22** You have isolated a cDNA clone encoding a protein of interest in a higher eukaryote. This cDNA clone is *not* cleaved by restriction endonuclease *EcoRI*. When this cDNA is used as a radioactive probe for blot hybridization analysis of *EcoRI*-digested genomic DNA, three radioactive bands are seen on the resulting Southern blot. Does this result indicate that the genome of the eukaryote in question contains three copies of the gene encoding the protein of interest?

**ANS:** No. The genome of the species in question may contain one, two, or three copies of the gene (or family of closely related genes) encoding this protein. The possibilities are as follows:

(1) One copy of the gene with two *EcoRI* cleavage sites located within intron sequences. (2) Two copies of the gene with one *EcoRI* cleavage site located within an intron sequence of one of the copies. (3) Three copies of the gene with no *EcoRI* cleavage site in any of the copies, that is, each copy present on a single *EcoRI* restriction fragment.

- 14.23** A linear DNA molecule is subjected to single and double digestions with restriction endonucleases, and the following results are obtained:

Enzymes	Fragment Sizes (in kb)
<i>EcoRI</i>	2.9, 4.5, 7.4, 8.0
<i>HindIII</i>	3.9, 6.0, 12.9
<i>EcoRI</i> and <i>HindIII</i>	1.0, 2.0, 2.9, 3.5, 6.0, 7.4

Draw the restriction map defined by these data.

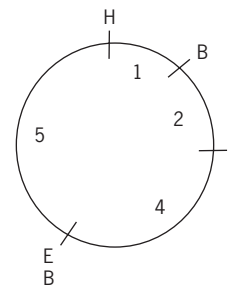
**ANS:**

- 14.24** A DNA molecule is subjected to single and double digestions with restriction enzymes, and the products are separated by gel electrophoresis. The results are as follows (fragment sizes are in kb):

	<i>EcoRI</i> and <i>HindIII</i>	<i>HindIII</i>	<i>BamHI</i>	<i>EcoRI</i> and <i>BamHI</i>	<i>HindIII</i> and <i>BamHI</i>
<i>EcoRI</i>	8	5	12	6	6
	4	4	6	4	5
		3		2	1

Draw the restriction map of this DNA molecule.

**ANS:**

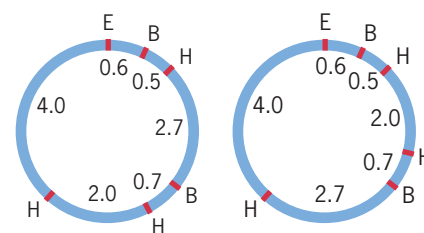


- 14.25** You are studying a circular plasmid DNA molecule of size 10.5 kilobase pairs (kb). When you digest this plasmid with restriction endonucleases *BamHI*, *EcoRI*, and *HindIII*, singly and in all possible combinations, you obtain linear restriction fragments of the following sizes:

Enzymes	Fragment Sizes (in kb)
<i>BamHI</i>	7.3, 3.2
<i>EcoRI</i>	10.5
<i>HindIII</i>	5.1, 3.4, 2.0
<i>BamHI</i> + <i>EcoRI</i>	6.7, 3.2, 0.6
<i>BamHI</i> + <i>HindIII</i>	4.6, 2.7, 2.0, 0.7, 0.5
<i>EcoRI</i> + <i>HindIII</i>	4.0, 3.4, 2.0, 1.1
<i>BamHI</i> + <i>EcoRI</i> + <i>HindIII</i>	4.0, 2.7, 2.0, 0.7, 0.6, 0.5

Draw a restriction map for the plasmid that fits your data.

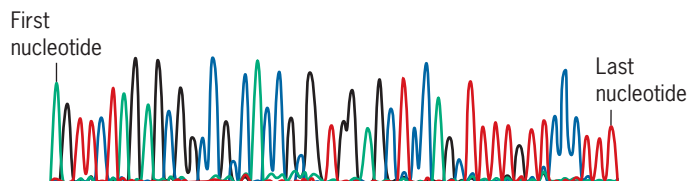
**ANS:** There are two possible restriction maps for these data as shown below:



Restriction enzyme cleavage sites for *BamHI*, *EcoRI*, and *HindIII* are denoted by B, E, and H, respectively. The numbers give distances in kilobase pairs.

- 14.26** The automated DNA sequencing machines utilize fluorescent dyes to detect the nascent DNA chains synthesized in the presence of the four dideoxy (ddX) chain terminators, each labeled with a different fluorescent dye. The dyes fluoresce at different wavelengths, which are recorded by a photocell as the products of the reactions are separated based on length by capillary gel electrophoresis (see Figure 14.17). In the standard sequencing reaction, the chains terminating with ddG fluoresce dark blue (peaks appear black in computer printout), those terminating with ddC fluoresce light blue, those terminating with ddA fluoresce green, and those terminating with ddT fluoresce red. The computer printout for the

sequence of a short segment of DNA is shown as follows.



What is the nucleotide sequence of the nascent strand of DNA?

What is the nucleotide sequence of the DNA template strand?

**ANS:** Nascent strand:

5'-AGTTCTAGAGCGGCCGCCACCGCGTGGAGCTCCAGCTTTTGTTCCCTTT-3'

Template strand:

3'-TCAAGATCTCGCCGGCGGTGGCGCACCTCGAGGTCGAAAAACAAGGAAA-5'

**14.27** Ten micrograms of a decanucleotide-pair *HpaI* restriction fragment were isolated from the double-stranded DNA chromosome of a small virus. Octanucleotide poly(A) tails were then added to the 3' ends of both strands using terminal transferase and dATP; that is,

5'-X X X X X X X X X X-3'

3'-X' X' X' X' X' X' X' X' X' X' X'-5'

↓ terminal transferase, dATP

5'-X X X X X X X X X X A A A A A A A A A-3'

3'-A A A A A A A A X' X' X' X' X' X' X' X' X' X'-5'

where X and X' can be any of the four standard nucleotides, but X' is always complementary to X.

The two complementary strands ("Watson" strand and "Crick" strand) were then separated and sequenced by the 2',3'-dideoxyribonucleoside triphosphate chain-termination method. The reactions were primed using a synthetic poly(T) octamer; that is,

Watson strand

3'-A A A A A A A A X' X' X' X' X' X' X' X' X' X'-5'

5'-T T T T T T T T-OH

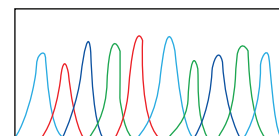
Crick strand

5'-X X X X X X X X X X A A A A A A A A A-3'

HO-T T T T T T T T-5'

Two DNA sequencing reactions were carried out. Reaction 1 contained the Watson strand template/primer shown above; reaction 2 contained the Crick strand template/primer. Both sequencing reactions contained DNA polymerase and all other substrates and components required for DNA synthesis *in vitro* plus the standard four 2',3'-dideoxyribonucleoside triphosphate

chain-terminators—ddGTP, ddCTP, ddATP, and ddTTP—each labeled with a different fluorescent dye. The dyes fluoresce at different wavelengths, which are recorded by a photocell as the products of the reactions are separated by capillary gel electrophoresis (see Figure 14.17). In the standard sequencing reaction, the chains terminating with ddG fluoresce dark blue (peaks appear black in the computer printouts), those terminating with ddC fluoresce light blue, those terminating with ddA fluoresce green, and those terminating with ddT fluoresce red. The computer printout for sequencing reaction 1, which contained the Watson strand as template, is shown as follows.



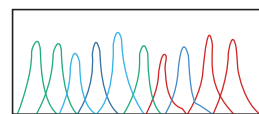
Nucleotide: 1 10

Draw the predicted computer printout for reaction 2, which contained the Crick strand as template, in the following box. Remember that all DNA synthesis occurs in the 5' → 3' direction and that the sequence of the nascent strand reads 5' to 3' from left to right in the printout.



Nucleotide: 1 10

**ANS:**



Nucleotide: 1 10

## CHAPTER 15

**15.1** Distinguish between a genetic map, a cytogenetic map, and a physical map. How can each of these types of maps be used to identify a gene by positional cloning?

**ANS:** Genetic map distances are determined by crossover frequencies. Cytogenetic maps are based on chromosome morphology or physical features of chromosomes. Physical maps are based on actual physical distances—the number of nucleotide pairs (0.34 nm per base pair)—separating genetic markers. If a gene or other DNA sequence of interest is shown to be located near a mutant gene, a specific band on a chromosome, or a particular DNA restriction fragment, that genetic or physical marker (mutation, band, or restriction fragment) can be used to initiate a chromosome walk to the gene of interest.

**15.2** In the technique of positional cloning, a researcher begins with a DNA library and selects a clone that is tightly linked to the gene of interest. That clone, or a

piece of it, is then used as a probe to isolate an overlapping clone from a different DNA library. The second clone is used to isolate a third overlapping clone from the first library, and so on, until the researcher has “walked” along the chromosome to the desired locus. (a) How can the researcher walk consistently in the same direction along the chromosome during the cloning process? (b) What could happen if a long repetitive DNA sequence such as a transposon was situated between the starting clone and the gene of interest?

**ANS:** (a) To ensure that the researcher “walks” in the same direction along the chromosome to the desired locus, it is necessary to check at each step in the process, usually by blot hybridization with subclones, which end of the clone used as a probe overlaps with the next recovered clone. (b) A long repetitive sequence could present a problem in chromosome walking because if that sequence is used as a probe to isolate a new clone, it will hybridize with restriction fragments from all over the genome, and thereby thwart the effort to walk systematically in one direction along a single chromosome. To overcome this problem, a researcher would have to “jump over” the repetitive element and use a piece of unique DNA beyond the element as a probe in the next step of the walking procedure.

**15.3** What is a contig? What is an RFLP? What is a VNTR? What is an STS? What is an EST? How is each of these used in the construction of chromosome maps?

**ANS:** A contig (*contiguous clones*) is a physical map of a chromosome or part of a chromosome prepared from a set of overlapping genomic DNA clones. An RFLP (*restriction fragment length polymorphism*) is a variation in the length of a specific restriction fragment excised from a chromosome by digestion with one or more restriction endonucleases. A VNTR (*variable number tandem repeat*) is a short DNA sequence that is present in the genome as tandem repeats and in highly variable copy number. An STS (*sequence tagged site*) is a unique DNA sequence that has been mapped to a specific site on a chromosome. An EST (*expressed sequence tag*) is a cDNA sequence—a genomic sequence that is transcribed. Contig maps permit researchers to obtain clones harboring genes of interest directly from DNA Stock Centers—to “clone by phone.” RFLPs are used to construct the high-density genetic maps that are needed for positional cloning. VNTRs are especially valuable RFLPs that are used to identify multiple sites in genomes. STSs and ESTs provide molecular probes that can be used to initiate chromosome walks to nearby genes of interest.

**15.4** The following is a Southern blot of *EcoRI*-digested DNA of rye plants from two different inbred lines, A and B. Developed autoradiogram I shows the bands resulting from probing the blot with <sup>32</sup>P-labeled cDNA1. Autoradiogram II shows the same Southern blot after it was stripped of probe and reprobed with <sup>32</sup>P-labeled cDNA2.

	I		II	
	A	B	A	B
a1	—	—	b1	—
			b2	—
a2		—		
a3		—	b3	—
a4	—			

(a) Which bands would you expect to see in the autoradiogram of a similarly probed Southern blot prepared using *EcoRI*-digested DNA from F<sub>1</sub> hybrid plants produced by crossing the two inbred lines? (b) What can you conclude about the gene(s) represented by band a1 on blot I in the two inbreds? (c) The F<sub>1</sub> plants were crossed to plants possessing only bands a1, a4, and b3. DNA was isolated from several individual progeny and digested with *EcoRI*. The resulting DNA fragments were separated by gel electrophoresis, transferred to a nylon membrane, and hybridized with radioactive cDNA1 and cDNA2 probes. The following table summarizes the bands present in autoradiograms obtained using DNA from individual progeny.

Plant No.	Bands Present						
	a1	a2	a3	a4	b1	b2	b3
1	+	+	+	+			+
2	+	+	+	+			+
3	+	+	+	+			+
4	+	+	+	+			+
5	+	+	+	+	+	+	+
6	+			+	+	+	+
7	+			+	+	+	+
8	+			+	+	+	+
9	+			+	+	+	+
10	+			+			+

Interpret these data. Do the data provide evidence for RFLPs? At how many loci? Are any of the RFLPs linked? If so, what are the linkage distances defined by the data?

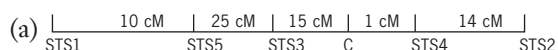
**ANS:** (a) a1, a2, a3, a4, b1, b2, and b3. (b) Band a1 represents a locus whose DNA is homologous to cDNA1. Since the marker is not polymorphic in the parents used, it cannot be mapped in this cross. (c) The cDNA1 probe detects one RFLP locus with alleles that are visualized as band a4 and bands a2/a3. The cDNA2 detects a second RFLP

locus with alleles that are visualized as band b3 and bands b1/b2. The two loci are linked with 20% recombination observed in this cross.

- 15.5 As part of the Human Genome Mapping Project, you are trying to clone a gene involved in colon cancer. Your first step is to localize the gene using RFLP markers. In the following table, RFLP loci are defined by STS number (e.g., STS1), and the gene for colon cancer is designated *C*.

Loci	% Recombination	Loci	% Recombination
<i>C</i> , STS1	50	STS1, STS5	10
<i>C</i> , STS2	15	STS2, STS3	30
<i>C</i> , STS3	15	STS2, STS4	14
<i>C</i> , STS4	1	STS2, STS5	50
<i>C</i> , STS5	40	STS3, STS4	16
STS1, STS2	50	STS3, STS5	25
STS1, STS3	35	STS4, STS5	41
STS1, STS4	50		

(a) Given the percentage recombination between different RFLP loci and the gene for colon cancer shown in the table, draw a genetic map showing the order and genetic distances between adjacent RFLP markers and the gene for colon cancer. (b) Given that the human genome contains approximately  $3.3 \times 10^9$  base pairs of DNA and that the human genetic map contains approximately 3300 cM, approximately how many base pairs of DNA are located along the stretch of chromosome defined by this RFLP map? (*Hint*: First figure how many base pairs of DNA are present per cM in the human genome.) (c) How many base pairs of DNA are present in the region between the colon cancer gene and the nearest STS?

**ANS:** (a) 

(b)  $3.3 \times 10^9 \text{ bp} / 3.3 \times 10^3 \text{ cM} = 1 \times 10^6 \text{ bp/cM}$ . The total map length is 65 cM, which equates to about  $65 \times 10^6$  or 65 million bp.

(c) The cancer gene (*C*) and STS4 are separated by 1 cM or about 1 million base pairs.

- 15.6 What are STRs? Why are they sometimes called microsatellites?

**ANS:** STRs are polymorphic tandem repeats of sequences of only two to four nucleotide pairs. They are called microsatellites because they are short in length and are components of the highly repetitive satellite DNAs of eukaryotes (see Chapter 9).

- 15.7 You have cloned a previously unknown human gene. What procedure will allow you to position this gene on the cytological map of the human genome without performing any pedigree analyses? Describe how you would carry out this procedure.

**ANS:** With a clone of the gene available, fluorescent *in situ* hybridization (FISH) can be used to determine which human chromosome carries the gene and to localize the gene on the chromosome. Single-stranded copies of the clone are coupled to a fluorescent probe and hybridized to denatured DNA in chromosomes spread on a slide. After hybridization, free probe is removed by washing, and the location of the fluorescent probe is determined by photography using a fluorescence microscope (see Focus on: *In Situ* Hybridization).

- 15.8 You have identified a previously unknown human EST. What must be done before this new EST can be called an STS?

**ANS:** An EST must be placed on the physical map of a chromosome before it can be called an STS. If it is also positioned on the genetic map of the chromosome, then it is called an anchor marker.

- 15.9 VNTRs and STRs are specific classes of polymorphisms. What is the difference between a VNTR and an STR?

**ANS:** Variable number tandem repeats (VNTRs) are composed of repeated sequences of 10–80 nucleotide pairs, and short tandem repeats (STRs) are composed of repeated sequences of 2–10 nucleotide pairs.

- 15.10 An RFLP and a mutant allele that causes albinism in humans cannot be shown to be separated by recombination based on pedigree analysis or by radiation hybrid mapping. Do these observations mean that the RFLP occurs within or overlaps the gene harboring the mutation that causes albinism? If so, why? If not, why not?

**ANS:** The resolution of genetic mapping in humans is quite low—in the range of 1–10 million base pairs. Radiation hybrid mapping provides higher resolution—to about 50 kb. However, even with 50-kb resolution, there could be several genes separating the RFLP from the mutation responsible for albinism.

- 15.11 A cloned 6-kb fragment of DNA from human chromosome 9 contains a single site recognized by the restriction enzyme *Eco*RI. This cloned fragment is demarcated by sites for the restriction enzyme *Bam*HI. There are no other *Bam*HI recognition sites within the clone. A researcher has collected DNA samples from 10 people. He digests each sample with a combination of *Eco*RI and *Bam*HI enzymes. The doubly digested DNA is then fractionated by gel electrophoresis and blotted to a membrane. After fixing the DNA to the membrane, the researcher hybridizes it with a radioactive probe made from the entire cloned *Bam*HI fragment. The autoradiogram obtained by exposing an X-ray film to this membrane yielded the following results. Three of the DNA samples contained a 4-kb fragment and a 2-kb fragment that hybridize with the probe, three of the DNA samples contained a 6-kb DNA fragment that hybridizes with the probe, and four of the DNA samples contain 6-, 4-, and 2-kb DNA fragments that hybridize with the probe.



What has this analysis revealed? What are the genotypes of the three different types of DNA samples?

**ANS:** The results of this analysis reveal that the *Eco*RI cleavage site within the original 6-kb *Bam*HI clone is polymorphic—that is, it is present in some chromosomes 9 but absent in others. We can represent these two types of chromosomes 9 as *BEB* (with the *Eco*RI site between flanking *Bam*HI sites) and *B-B* (without the *Eco*RI site). The first three samples came from people who were homozygous for the *BEB* version of chromosome 9, the next three samples came from people who were homozygous for the *B-B* version, and the last four samples came from people who were heterozygous for the two versions—that is, they had the genotype *BEB/B-B*. In the human population that was sampled, the *Eco*RI site is therefore the basis for a restriction fragment length polymorphism (RFLP).

**15.12** Both an RFLP and a mutation that causes deafness in humans map to the same location on the same chromosome. How can you determine whether or not the RFLP overlaps with the gene containing the deafness mutation?

**ANS:** You can start a chromosome walk using the hybridization probe that detects the RFLP. However, if a physical map of this region of the chromosome already exists (see Figure 15.5), a chromosome walk might not be necessary. cDNAs can be used to locate candidate genes in the region covered by the chromosome walk, and the sequences of genes in individuals with this form of inherited deafness can be compared with the sequences of homologous genes of individuals with normal hearing, looking for changes that would be expected to cause a loss of gene function. The overall process is illustrated in Figure 15.6.

**15.13** What were the goals of the Human Genome Project? What impact has achieving these goals had on the practice of medicine to date? What are some of the predicted future impacts? What are some of the possible misuses of human genome data?

**ANS:** The goals of the Human Genome Project were to prepare genetic and physical maps showing the locations of all the genes in the human genome and to determine the nucleotide sequences of all 24 chromosomes in the human genome. These maps and nucleotide sequences of the human chromosomes helped scientists identify mutant genes that result in inherited diseases. Hopefully, the identification of these mutant disease genes will lead to successful treatments, including gene therapies, for at least some of these diseases in the future. Potential misuses of these data include invasions of privacy by governments and businesses—especially employment agencies and insurance companies. Individuals must not be denied educational opportunities, employment, or insurance because of inherited diseases or mutant genes that result in a predisposition to mental or physical abnormalities.

**15.14** What difficulty does repetitive DNA pose for the assembly of whole genome shotgun sequences by computer analysis?

**ANS:** Repetitive DNA can make it difficult to assemble long stretches of DNA sequence from the sequences of DNA fragments that contain these sequences. The reason is that in the computer analysis, a repetitive sequence in one DNA fragment will match with the same repetitive sequence in another DNA fragment even if the two DNA fragments are not contiguous in the genome. The effort to determine which short DNA fragments are authentic neighbors (contiguous to each other) can therefore be thrown off by these spurious matches.

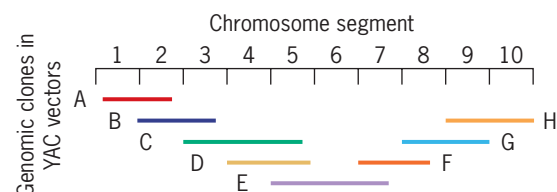
**15.15** Which type of molecular marker, RFLP or EST, is most likely to mark a disease-causing mutant gene in humans? Why?

**ANS:** An EST is more likely than an RFLP to occur in a disease-causing human gene. All ESTs correspond to expressed sequences in a genome. RFLPs occur throughout a genome, in both expressed and unexpressed sequences. Because less than 2 percent of the human genome encodes proteins, most RFLPs occur in noncoding DNA.

**15.16** Bacteriophage  $\Phi$ X174 contains 11 genes in a genome of 5386 bp; *E. coli* has a predicted 4288 genes in a genome of about 4.639 kb; *S. cerevisiae* has about 6000 genes in a genome of size 12.1 mb; *C. elegans* has about 19,000 genes present in a genome of about 100 mb; and *H. sapiens* has an estimated 22,000 genes in its 3000-mb genome. Which genome has the highest gene density? Which genome has the lowest gene density? Does there appear to be any correlation between gene density and developmental complexity? If so, describe the correlation.

**ANS:** The bacteriophage  $\Phi$ X174 genome has the highest gene density—one gene per 490 nucleotide pairs. The human genome has the lowest gene density—about one gene per 100 kb. In the species mentioned in this question, there is a striking correlation between genome size and developmental complexity. With some exceptions, including species with polyploid genomes, there does appear to be a rough correlation between genome size and developmental complexity.

**15.17** A contig map of one segment of chromosome 3 of *Arabidopsis* is as follows.



(a) If an EST hybridizes with genomic clones C, D, and E, but not with the other clones, in which segment of chromosome 3 is the EST located? (b) If a clone of gene *ARA* hybridizes only with genomic clones C and D, in

which chromosome segment is the gene located? (c) If a restriction fragment hybridizes with only one of the genomic clones shown above, in which chromosome segment(s) could the fragment be located?

**ANS:** (a) Segment 5; (b) segment 4; (c) segment 1, 6, or 10.

**15.18** Eight human–Chinese hamster radiation hybrids were tested for the presence of six human ESTs designated A through F. The results are shown in the following table, where a plus indicates that a marker was present and a minus indicates that it was absent.

	Radiation hybrid							
	1	2	3	4	5	6	7	8
Marker A	-	+	-	-	+	+	-	+
B	+	-	+	-	-	+	-	+
C	-	+	+	+	-	-	-	+
D	+	-	+	+	-	-	+	-
E	+	-	+	+	-	-	+	-
F	-	+	-	+	+	+	+	-

Based on these data, do any of the ESTs appear to be closely linked? Which ones? What would be needed for you to be more certain of your answer?

**ANS:** EST markers D and E appear to be closely linked. The eight human–Chinese hamster radiation hybrids contain either both D and E or neither marker. More radiation hybrids would need to be tested for the presence of these ESTs to obtain convincing evidence of this linkage.

**15.19** What is the major advantage of gene chips as a microarray hybridization tool?

**ANS:** The major advantage of gene chips as a microarray hybridization tool is that a single gene chip can be used to quantify thousands of distinct nucleotide sequences simultaneously. The gene-chip technology allows researchers to investigate the levels of expression of a large number of genes more efficiently than was possible using earlier microarray procedures.

**15.20** What major advantage does the green fluorescent protein of the jellyfish have over other methods for studying protein synthesis and localization?

**ANS:** The green fluorescent protein (GFP) can be used to study protein localization and movement over time in living cells. Most other procedures for studying protein localization require that cells be permeabilized and/or fixed and exposed to antibodies coupled to radioactive or fluorescent compounds prior to visualization. As a result, these procedures only provide information about the location of a protein at a single time point. In contrast, GFP-tagged proteins can be used to study the synthesis and movement of proteins in living cells over time (hours to days).

**15.21** You are given chromosome-specific cDNA libraries for all 24 human chromosomes. How might these libraries be used to study chromosome evolution in primates?

**ANS:** The DNA sequences in human chromosome-specific cDNA libraries can be coupled to fluorescent dyes and hybridized *in situ* to the chromosomes of other primates. The hybridization patterns can be used to detect changes in genome structure that have occurred during the evolution of the various species of primates from common ancestors (see Figure 6.4). Such comparisons are especially effective in detecting new linkage relationships resulting from translocations and centric fusions.

**15.22** Of the cereal grass species, only maize contains two copies of each block of linked genes. What does this duplication of sets of maize genes indicate about the origin of this agronomically important species?

**ANS:** The presence of two copies of each block of genes in the corn genome indicates that maize has evolved from a tetraploid ancestor. The presence of one set of genes primarily in the large chromosomes and the second set largely in the small chromosomes suggests that maize has evolved from an allotetraploid (see Chapter 6) produced by combining the diploid genomes of two ancestral cereal grass species.

**15.23** Five human genomic DNA clones present in PAC vectors were tested by hybridization for the presence of six sequence-tagged sites designated STS1 through STS6. The results are given in the following table: a plus indicates the presence of the STS, and a minus indicates the absence of the STS.

	STS					
	1	2	3	4	5	6
YAC clone A	+	-	+	+	-	-
B	+	-	-	-	+	-
C	-	-	+	+	-	+
D	-	+	-	-	+	-
E	-	-	+	-	-	+

(a) What is the order of the STS sites on the chromosome?

(b) Draw the contig map defined by these data.

**ANS:** (a) Order of STS sites: 2-5-1-4-3-6.

(b) STS markers: 2 5 1 4 3 6



**15.24** The complete sequences of six mitochondrial genomes of *H. neanderthalensis* have been available for some time; the first *H. neanderthalensis* mtDNA sequence was published in 2008. How similar are the sequences of the mtDNAs of *H. neanderthalensis* and *H. sapiens*? Are the genomes similar in size? Is the amount of diversity observed in the mtDNAs of Neanderthals and humans the same? If not, what might this tell us about the sizes of Neanderthal and human populations? How many genes are present in the *H. neanderthalensis* mitochondrial

genome? How many of these genes encode proteins? How many specify structural RNA molecules? Are there any pseudogenes in *H. neanderthalensis* mtDNA? All of these questions can be answered by visiting the <http://www.ncbi.nlm.nih.gov/> web site.

**ANS:** The mtDNAs of *Homo neanderthalensis* and *H. sapiens* are very similar, both in size and in nucleotide sequence. The *H. neanderthalensis* mtDNA contains 16,565 nucleotide pairs, whereas the *H. sapiens* mtDNA contains 16,569 nucleotide pairs, and the two DNAs exhibit 99 percent sequence identity. There is less sequence diversity in the mtDNAs of Neanderthals than in the mtDNAs of humans, which is consistent with Neanderthal populations being smaller than human populations. There are 37 genes in the Neanderthal mtDNA: 13 encode proteins and 24 specify structural RNAs (tRNAs and rRNAs). The Neanderthal mitochondrial genome does not contain any pseudogenes.

**15.25** Assume that you have just sequenced a small fragment of DNA that you had cloned. The nucleotide sequence of this segment of DNA is as follows.

```
aagtagtcgaaaccgaattccgtagaacaactcgcacgctccggtttc
gtgttgcaacaaaataggcattcccatcgcggcagttagaatcaccga
gtgccagagtcacgttcgtaagcagggcgcagttacagggcagca
gaaaaatcgattgaacagaaaatggctggcggtaaagcaggcaagga
ttcgggcaaggccaaggcgaaggcggatcgcgttccgcgcgcccggg
```

In an attempt to learn something about the identity or possible function of this DNA sequence, you decide to perform a BLAST (nucleotide blast) search on the NCBI web site (<http://www.ncbi.nlm.nih.gov>). Paste or type this sequence into the query sequence box. Run the search and examine the sequences most closely related to your query sequence. Are they coding sequences? What proteins do they encode? Repeat the BLAST search with only half of your sequence as the query sequence. Do you still identify the same sequences in the databases? If you use one-fourth of your sequence as a query, do you still retrieve the same sequences? What is the shortest DNA sequence that you can use as a query and still identify the same sequences in the databanks?

**ANS:** All of the sequences identified by the megablast search encode histone H2a proteins. The query sequence is identical to the coding sequence of the *Drosophila melanogaster* histone *H2aV* gene (a member of the gene family encoding histone H2a proteins). The query sequence encodes a *Drosophila* histone H2a polypeptide designated variant V. The same databank sequences are identified when one-half or one-fourth of the given nucleotide sequence is used as the query in the megablast search. Query sequences as short as 15–20 nucleotides can be used to identify the *Drosophila* gene encoding the histone H2a variant. However, the results will vary depending on the specific nucleotide sequence used as the query sequence.

**15.26** The NCBI web site (<http://www.ncbi.nlm.nih.gov>) can also be used to search for protein sequences. Instead of performing a BLAST search with a nucleic acid query, one performs a protein blast with a polypeptide (amino acid sequence) query. Assume that you have the following partial sequence of a polypeptide:

```
GYDVEKNNNSRIKLGKLSLVSKGILVQTKGT
GASGSFKLNKKAASGEAKPQAKKAGAAKA
```

Go to the NCBI web site and access the BLAST tool. Then click on protein blast and enter the query sequence in the box at the top. Then click BLAST. What is the identity of your query sequence?

**ANS:** Your query sequence is a portion of histone H1.2 of the mouse (*Mus musculus*). This portion of the mouse histone H1.2 polypeptide differs by one amino acid from the corresponding part of the human H1 histone.

**15.27** The sequence of a gene in *Drosophila melanogaster* that encodes a histone H2A polypeptide is as follows:

```
aagtagtcgaaaccgaattccgtagaacaactcgcacgctccggtttc
gtgttgcaacaaaataggcattcccatcgcggcagttagaatcacc
gagtgcccagagtcacgttcgtaagcagggcgcagttacagggcagcag
aaaaatcgattgaacagaaaatggctggcggtaaagcaggcaagg
attcgggcaaggccaaggcgaaggcgggtatcgcggtccgcgcg
cgcgggtcttcagttcccggtgggtcgcacatcgcacatctcaag
agccgcaactacgtcacatggacgcgctggagccactgcagccgtg
tactccgctgccatattggaatacctgaccgcccaggtcctggagtt
ggcaggcaacgcacatcgaaggacttgaagtgaacgtatcactcc
tcgccacttacagctgccattcgcggagacgaggagctggacag
cctgatcaaggcaaccatcgcgtggtggcggtgctattccgcacata
cacaagtcgctgatcggcaaaaaggaggaaacgggtgcaggatccgc
agcgggaaggggcaacgtcattctgctcagggcctaactaagccagtcgg
caatcggacgccttcgaaacatgcaacactaatgtttaattcagatt
cagcagagacaagctaaaacaccgacgagttgtaataattctgtgcg
ccagcatatattcttatatacaacgtaatacataattatgtaattctagca
tctcccaactcacaatacaatacaaaaaatacaaacacacaaaaac
gtatttaccgcacgcatccttggcgagggttgagtatgaaacaaa
aacaacttaatttagagcaagtaattacacgaataaatttaataa
aaaaactataataaaaacgcc.
```

Let's use the translation software available on the Internet at <http://www.expasy.org/tools/dna.html> to translate this gene in all six possible reading frames and see which reading frame specifies histone H2A. Just type or paste the DNA sequence in the "ExPASy Translate" tool box and click TRANSLATE SEQUENCE. The results will show the products of translation in all six reading frames with **Met's** and **Stop's** boldfaced to highlight potential open-reading frames. Which reading frame specifies histone H2A?

**ANS:** Reading frame 5' → 3' number 1 has a large open reading frame with a methionine codon near the 5' end. You can verify that this is the correct reading frame by using the predicted translation product as a query to search one of the protein databases (see Problem 15.26).

## CHAPTER 16

**16.1** What are CpG islands? Of what value are CpG islands in positional cloning of human genes?

**ANS:** CpG islands are clusters of cytosines and guanines that are often located just upstream (5') from the coding regions of human genes. Their presence in nucleotide sequences can provide hints as to the location of genes in human chromosomes.

**16.2** Why is the mutant gene that causes Huntington's disease called *huntingtin*? Why might this gene be renamed in the future?

**ANS:** The gene was named *huntingtin* after the disease that it causes when defective. The gene will probably be renamed after the function of its gene product has been determined.

**16.3** How was the nucleotide sequence of the *CF* gene used to obtain information about the structure and function of its gene product?

**ANS:** The *CF* gene was identified by map position-based cloning, and the nucleotide sequences of *CF* cDNAs were used to predict the amino acid sequence of the *CF* gene product. A computer search of the protein data banks revealed that the *CF* gene product was similar to several ion channel proteins. This result focused the attention of scientists studying cystic fibrosis on proteins involved in the transport of salts between cells and led to the discovery that the *CF* gene product was a transmembrane conductance regulator—now called the CFTR protein.

**16.4** How might the characterization of the *CF* gene and its product lead to the treatment of cystic fibrosis by somatic-cell gene therapy? What obstacles must be overcome before cystic fibrosis can be treated successfully by gene therapy?

**ANS:** Once the function of the *CF* gene product has been established, scientists should be able to develop procedures for introducing wild-type copies of the *CF* gene into the appropriate cells of cystic fibrosis patients to alleviate the devastating effects of the mutant gene. A major obstacle to somatic-cell gene-therapy treatment of cystic fibrosis is the size of the *CF* gene—about 250 kb, which is too large to fit in the standard gene transfer vectors. Perhaps a shortened version of the gene constructed from the *CF* cDNA—about 6.5 kb—can be used in place of the wild-type gene. A second major obstacle is getting the transgene into enough of the target cells of the cystic fibrosis patient to alleviate the symptoms of the disease. A third challenge is to develop an expression vector containing the gene that will result in long-term expression of the introduced gene in transgenic cells. Another concern is how to avoid possible side effects caused by overexpression or inappropriate expression of the transgene in cystic fibrosis patients. Despite these obstacles, many scientists are optimistic that cystic fibrosis will be effectively treated by somatic-cell gene therapy in the future.

**16.5** Myotonic dystrophy (MD), occurring in about 1 of 8000 individuals, is the most common form of muscular dystrophy in adults. The disease, which is characterized by progressive muscle degeneration, is caused by a dominant mutant gene that contains an expanded CAG repeat region. Wild-type alleles of the *MD* gene contain 5–30 copies of the trinucleotide. Mutant *MD* alleles contain 50 to over 2000 copies of the CAG repeat. The complete nucleotide sequence of the *MD* gene is available. Design a diagnostic test for the mutant gene responsible for myotonic dystrophy that can be carried out using genomic DNA from newborns, fetal cells obtained by amniocentesis, and single cells from eight-cell pre-embryos produced by *in vitro* fertilization.

**ANS:** Oligonucleotide primers complementary to DNA sequences on both sides (upstream and downstream) of the CAG repeat region in the *MD* gene can be synthesized and used to amplify the repeat region by PCR. One primer must be complementary to an upstream region of the template strand, and the other primer must be complementary to a downstream region of the nontemplate strand. After amplification, the size(s) of the CAG repeat regions can be determined by gel electrophoresis (see Figure 16.2). Trinucleotide repeat lengths can be measured by including repeat regions of known length on the gel. If fewer than 30 copies of the trinucleotide repeat are present on each chromosome, the newborn, fetus, or pre-embryo is homozygous for a wild-type *MD* allele or heterozygous for two different wild-type *MD* alleles. If more than 50 copies of the repeat are present on each of the homologous chromosomes, the individual, fetus, or cell is homozygous for a dominant mutant *MD* allele or heterozygous for two different mutant alleles. If one chromosome contains less than 30 copies of the CAG repeat and the homologous chromosome contains more than 50 copies, the newborn, fetus, or pre-embryo is heterozygous, carrying one wild-type *MD* allele and one mutant *MD* allele.

**16.6** In humans, the absence of an enzyme called purine nucleoside phosphorylase (PNP) results in a severe T-cell immunodeficiency similar to that of severe combined immunodeficiency disease (SCID). PNP deficiency exhibits an autosomal recessive pattern of inheritance, and the gene encoding human PNP has been cloned and sequenced. Would PNP deficiency be a good candidate for treatment by gene therapy? Design a procedure for the treatment of PNP deficiency by somatic-cell gene therapy.

**ANS:** Yes. A somatic-cell gene therapy procedure similar to that used for X-linked SCID (see Figure 16.7) might be effective in treating purine nucleoside phosphorylase (PNP) deficiency. White blood cells could be isolated from the patient, transfected with a vector carrying a wild-type *PNP* gene, grown in culture and assayed for the expression of the *PNP* transgene, and then infused back into the patient after the expression of the transgene had been verified.

**16.7** Human proteins can now be produced in bacteria such as *E. coli*. However, one cannot simply introduce a human gene into *E. coli* and expect it to be expressed. What steps must be taken to construct an *E. coli* strain that will produce a mammalian protein such as human growth hormone?

**ANS:** The transcription initiation and termination and translation initiation signals or eukaryotes differ from those of prokaryotes such as *E. coli*. Therefore, to produce a human protein in *E. coli*, the coding sequence of the human gene must be joined to appropriate *E. coli* regulatory signals—promoter, transcription terminator, and translation initiator sequences. Moreover, if the gene contains introns, they must be removed or the coding sequence of a cDNA must be used, because *E. coli* does not possess the spliceosomes required for the excision of introns from nuclear gene transcripts. In addition, many eukaryotic proteins undergo posttranslational processing events that are not carried out in prokaryotic cells. Such proteins are more easily produced in transgenic eukaryotic cells growing in culture.

**16.8** You have constructed a synthetic gene that encodes an enzyme that degrades the herbicide glyphosate. You wish to introduce your synthetic gene into *Arabidopsis* plants and test the transgenic plants for resistance to glyphosate. How could you produce a transgenic *Arabidopsis* plant harboring your synthetic gene by *A. tumefaciens*-mediated transformation?

**ANS:** You would first construct a chimeric gene containing your synthetic gene fused to a plant promoter such as the 35S promoter of cauliflower mosaic virus and a plant transcription termination and polyadenylation signal such as the one from the *nos* gene of the Ti plasmid. This chimeric gene would then be inserted into the T-DNA of a Ti plasmid carrying a dominant selectable marker gene (e.g., 35S/NPTII/*nos*, which confers resistance to kanamycin to host cells) and introduced into *Agrobacterium tumefaciens* cells by transformation. Tissue explants from *Arabidopsis* plants would be co-cultivated with *A. tumefaciens* cells harboring the recombinant Ti plasmid, and plant cells that carry T-DNAs inserted into their chromosomes would be selected by growth on medium containing the appropriate selective agent (e.g., kanamycin). Transgenic plants would then be regenerated from the transformed cells and tested for resistance to glyphosate.

**16.9** A human STR locus contains a tandem repeat (TAGA)<sub>*n*</sub>, where *n* may be any number between 5 and 15. How many alleles of this locus would you expect to find in the human population?

**ANS:** Eleven, ranging in multiples of 3, from 15 to 45 nucleotides long.

**16.10** A group of bodies are found buried in a forest. The police suspect that they may include the missing Jones family (two parents and two children). They extract DNA from

bones and examine (using PCR) genes *A* and *B*, which are known to contain tandem triplet repeats of variable length. They also analyze DNA from two other men. The results are shown below where the numbers indicate the number of copies of a tandem repeat in a particular allele; for example, male 1 has one allele with 8 and another allele with 9 copies of a tandem repeat in gene *A*.

	Gene <i>A</i>	Gene <i>B</i>
Male 1	8/9	5/7
Male 2	6/8	5/5
Male 3	7/10	7/7
Woman	8/8	3/5
Child 1	7/8	5/7
Child 2	8/8	3/7

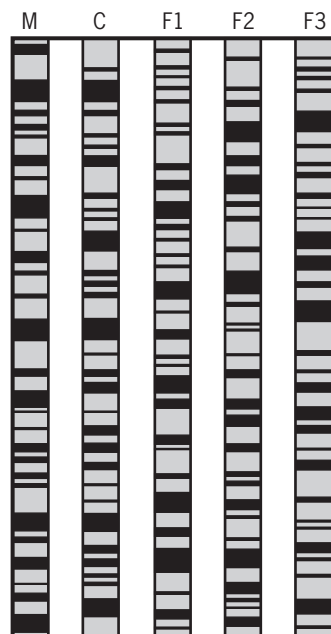
Could the woman have been the mother of both children? Why or why not? Which man, if any, could have been the father of child 1?

**ANS:** The woman could be the mother of both children, having passed alleles *A* 8 and *B* 5 to child 1 and *A* 8 and *B* 3 to child 2. Male 3 could have been the father of child 1. If so, he contributed alleles *A* 7 and *B* 7.

**16.11** DNA profiles have played central roles in many rape and murder trials. What is a DNA profile? What roles do DNA profiles play in these forensic cases? In some cases, geneticists have been concerned that DNA profile data were being used improperly. What were some of their concerns, and how can these concerns be properly addressed?

**ANS:** DNA profiles are the specific patterns (1) of peaks present in electropherograms of chromosomal STRs or VNTRs amplified by PCR using primers tagged with fluorescent dyes and separated by capillary gel electrophoresis (see Figures 16.11 and 16.12) or (2) of bands on Southern blots of genomic DNAs that have been digested with specific restriction enzymes and hybridized to appropriate STR or VNTR sequences (see Figure 16.10). DNA profiles, “like” epidermal fingerprints, are used as evidence for identity or nonidentity in forensic cases. Geneticists have expressed concerns about the statistical uses of DNA profile data. In particular, they have questioned some of the methods used to calculate the probability that DNA from someone other than the suspect could have produced an observed profile. These concerns have been based in part on the lack of adequate DNA profile databases for various human subpopulations and the lack of precise information about the amount of variability in DNA profiles for individuals of different ethnic backgrounds. These concerns have been addressed by the acquisition of data on profile frequencies in different populations and ethnic groups from throughout the world.

- 16.12** The DNA profiles shown in this problem were prepared using genomic DNA from blood cells obtained from a woman, her daughter, and three men who all claim to be the girl's father.



Based on the DNA profiles, what can be determined about paternity in this case?

- ANS:** Neither F1 nor F2 could be the girl's biological father; only individual F3 could be the child's father.

- 16.13** Most forensic experts agree that profiles of DNA from blood samples obtained at crime scenes and on personal items can provide convincing evidence for murder convictions. However, the defense attorneys sometimes argue successfully that sloppiness in handling blood samples results in contamination of the samples. What problems would contamination of blood samples present in the interpretation of DNA profiles? Would you expect such errors to lead to the conviction of an innocent person or the acquittal of a guilty person?

- ANS:** Contamination of blood samples would introduce more variability into DNA profiles. This would lead to a lack of allelic matching of profiles obtained from the blood samples and from the defendant. Mixing errors would be expected to lead to the acquittal of a guilty person and not to the conviction of an innocent person. Only the mislabeling of samples could implicate someone who is innocent.

- 16.14** The Ti plasmid contains a region referred to as T-DNA. Why is this region called T-DNA, and what is its significance?

- ANS:** The T in T-DNA is an abbreviation for "transferred." The T-DNA region of the Ti plasmid is the segment that is transferred from the Ti plasmid of the bacterium to the chromosomes of the plant cells during *Agrobacterium tumefaciens*-mediated transformation.

- 16.15** The generation of transgenic plants using *A. tumefaciens*-mediated transformation often results in multiple sites of insertion. These sites frequently vary in the level of transgene expression. What approaches could you use to determine whether or not transgenic plants carry more than one transgene and, if so, where the transgenes are inserted into chromosomes?

- ANS:** Probing Southern blots of restriction enzyme-digested DNA of the transgenic plants with  $^{32}\text{P}$ -labeled transgene may provide evidence of multiple insertions but would not reveal the genomic location of the inserts. Fluorescence *in situ* hybridization (FISH) is a powerful procedure for determining the genomic location of gene inserts. FISH is used to visualize the location of transgenes in chromosomes.

- 16.16** Disarmed retroviral vectors can be used to introduce genes into higher animals including humans. What advantages do retroviral vectors have over other kinds of gene-transfer vectors? What disadvantages?

- ANS:** Disarmed retroviruses are lacking genes essential for reproduction in host cells but can still integrate into the DNA of the host cell in the proviral state. The retroviral genomes are small enough to allow them to be manipulated easily *in vitro* and yet will accept foreign DNA inserts of average gene size. The retroviruses contain strong promoters in their long terminal repeats that can be used to drive high levels of transcription of the foreign gene insert.

- 16.17** Transgenic mice are now routinely produced and studied in research laboratories throughout the world. How are transgenic mice produced? What kinds of information can be obtained from studies performed on transgenic mice? Does this information have any importance to the practice of medicine? If so, what?

- ANS:** Transgenic mice are usually produced by microinjecting the genes of interest into pronuclei of fertilized eggs or by infecting pre-implantation embryos with retroviral vectors containing the genes of interest. Transgenic mice provide invaluable tools for studies of gene expression, mammalian development, and the immune system of mammals. Transgenic mice are of major importance in medicine; they provide the model system most closely related to humans. They have been, and undoubtedly will continue to be, of great value in developing the tools and technology that will be used for human gene therapy in the future.

- 16.18** Two men claim to be the father of baby Joyce Doe. Joyce's mother had her CODIS STR DNA profile analyzed and was homozygous for allele 8 at the TPOX locus (allele 8 contains 8 repeats of the GAAT sequence at this polymorphic locus). Baby Joyce is heterozygous for alleles 8 and 11 at this locus. In an attempt to resolve the disputed paternity, the two men were tested for their STR DNA profiles at the TPOX locus on chromosome 2. Putative father 1 was heterozygous for alleles 8 and 11 at the

TPOX locus, and putative father 2 was homozygous for allele 11 at this locus. Can these results resolve this case of disputed paternity. If so, who is the biological father? If not, why not?

**ANS:** The results cannot resolve the case of disputed paternity. Baby Joyce received allele 8 at the TPOX locus from her mother. Given that baby Joyce is heterozygous for alleles 8 and 11 at the TPOX locus, she must have received allele 11 from her father. However, both of the putative fathers carry allele 11 and could have passed it on to baby Joyce.

**16.19** Many valuable human proteins contain carbohydrate or lipid components that are added posttranslationally. Bacteria do not contain the enzymes needed to add these components to primary translation products. How might these proteins be produced using transgenic animals?

**ANS:** Posttranslationally modified proteins can be produced in transgenic eukaryotic cells growing in culture or in transgenic plants and animals. Indeed, transgenic sheep have been produced that secrete human blood-clotting factor IX and  $\alpha$ 1-antitrypsin in their milk. These sheep were produced by fusing the coding sequences of the respective genes to a DNA sequence that encodes the signal peptide required for secretion and introducing this chimeric gene into fertilized eggs that were then implanted and allowed to develop into transgenic animals. In principle, this approach could be used to produce any protein of interest.

**16.20** Richard Meagher and coworkers have cloned a family of 10 genes that encode actins (a major component of the cytoskeleton) in *Arabidopsis thaliana*. The 10 actin gene products are similar, often differing by just a few amino acids. Thus, the coding sequences of the 10 genes are also very similar, so that the coding region of one gene will cross-hybridize with the coding regions of the other nine genes. In contrast, the noncoding regions of the 10 genes are quite divergent. Meagher has hypothesized that the 10 actin genes exhibit quite different temporal and spatial patterns of expression. You have been hired by Meagher to test this hypothesis. Design experiments that will allow you to determine the temporal and spatial pattern of expression of each of the 10 actin genes in *Arabidopsis*.

**ANS:** You can subclone the 3' noncoding regions (sequences between the translation-termination codons and the 3' termini of the transcripts) of the actin genes and use these sequences as gene-specific hybridization probes, after verifying their specificity (no cross-hybridization to the other genes in the gene family). This procedure has worked elegantly for the 15 tubulin genes and the 10 actin genes of *Arabidopsis*, because the transcript sequences are very divergent in the 5' and 3' noncoding regions. You cannot, of course, use intron sequences because they are excised during pre-mRNA processing. These 3' noncoding gene-specific hybridization probes

are then used to measure individual gene transcript levels in various organs and tissues of developing plants by either northern blot or *in situ* hybridization experiments. Alternatively, the 5' and 3' noncoding regions can be used to design gene-specific PCR primers, and reverse transcription PCR (RT-PCR) can be used to measure individual gene transcript levels in organs and tissues. Indeed, Meagher and colleagues have already used this approach to document the striking temporal and tissue-specific patterns of actin gene expression in *Arabidopsis*.

**16.21** The first transgenic mice resulted from microinjecting fertilized eggs with vector DNA similar to that diagrammed in Figure 16.15 except that it contained a promoter for the mammalian metallothionein gene linked to the *HGH* gene. The resulting transgenic mice showed elevated levels of HGH in tissues of organs other than the pituitary gland—for example, in heart, lung, and liver—and the pituitary gland underwent atrophy. How might the production of HGH in transgenic animals be better regulated, with expression restricted to the pituitary gland?

**ANS:** The vector described contains the HGH gene; however, it does not contain a mammalian HGH promoter that will regulate the expression of the transgene in the appropriate tissues. Construction of vectors containing a properly positioned mammalian HGH-promoter sequence should result in transgenic mice in which HGH synthesis is restricted to the pituitary gland.

**16.22** How do the reverse genetic approaches used to dissect biological processes differ from classical genetic approaches?

**ANS:** Classical genetic approaches use mutational dissection to probe the functions of genes. Mutant alleles that produce altered phenotypes are identified and used to investigate the functions of the wild-type alleles. Comparative molecular analyses of mutant and wild-type organisms sometimes allow researchers to determine the precise function of a gene. Reverse genetic approaches use the known nucleotide sequences of genes to design procedures to either produce null mutations in them or inhibit their expression. RNA interference protocols are used to block the expression of specific genes. T-DNA and transposon insertion protocols are used to produce null mutations (to “knock out” the function) of specific genes. Basically, in classical genetics, you start with mutant alleles and hope to uncover the wild-type gene function; in reverse genetics, you start with the wild-type gene and generate mutant alleles.

**16.23** How can RNAi gene silencing be used to determine the function of genes?

**ANS:** RNAi involves the use of double-stranded RNAs, where one strand is complementary to the mRNA and the other strand is equivalent to the mRNA, to silence the expression of target genes. RNAi makes use of the RNA-induced silencing complex (RISC) to block gene expression (see Figure 16.23).

**16.24** How do insertional mutagenesis approaches differ from other reverse genetic approaches?

**ANS:** Insertional mutagenesis approaches produce mutant alleles—usually null alleles, or “knockouts,”—by the insertion of foreign DNA into genes. Other reverse genetic approaches, for example, RNA interference, inhibit the expression of the genes but leave them structurally intact.

**16.25** Insertional mutagenesis is a powerful tool in both plants and animals. However, when performing large-scale insertional mutagenesis, what major advantage do plants have over animals?

**ANS:** Plants have an advantage over animals in that once insertional mutations are induced they can be stored for long periods of time and distributed to researchers as dormant seeds.

**16.26** We discussed the unfortunate effects of insertional mutagenesis in the four boys who developed leukemia after treatment of X-linked severe combined immunodeficiency disease by gene therapy. How might this consequence of gene therapy be avoided in the future? Do you believe that the use of somatic-cell gene therapy to treat human diseases can ever be made 100 percent risk-free? Why? Why not?

**ANS:** The vectors used in somatic-cell gene therapy must not insert themselves preferentially into important genes, especially proto-oncogenes. Ideally, the vectors should insert themselves into unessential regions of the human genome. However, such vectors may not exist. At the minimum, however, vectors should be used that insert into the genome at random sites so that their chance of inserting into an important regulatory gene such as a proto-oncogene is very low. Gene therapy will probably never be completely risk-free, because anomalous insertion events will always occur at some low frequency. No biological process is 100 percent accurate. However, the potential benefits of gene therapy must outweigh the potential risks before the procedure will become an accepted tool for treating inherited diseases.

**16.27** One strand of a gene in *Arabidopsis thaliana* has the following nucleotide sequence:

```
atgagtgacgggaggaggaagaagagcgtgaacggagggt
gcaccggcgcaacaatcttggatgatcggagatctagtcttcgga
agttgaagcttccaccggctgggaaacgagctgttatcaagagtgc
cgatatgaaagatgatatgcaaaaggaagctatcgaaatcgccatct
ccgcgtttgagaagtacagtgtggagaaggatagctgagaatataa
agaaggagtttgacaagaacatggtgctacttggcattgcattgttg
tcgcaacttggttcttatgtaacgatgagacaaccatttcgttacttct
acctgaccagaagctgtgctgctcttcaagtcgggttaa
```

The function(s) of this gene is still uncertain. (a) How might insertional mutagenesis be used to investigate the function(s) of the gene? (b) Design an experiment using RNA interference to probe the function(s) of the gene.

**ANS:** (a) You would first want to check the Salk Institute’s Genome Analysis Laboratory web site to see if a T-DNA or transposon insertion has already been identified in this gene (see Problem 16.28). If so, you can simply order seeds of the transgenic line from the *Arabidopsis* Biological Resource Center at Ohio State University. If no insertion is available in the gene, you can determine where it maps in the genome and use transposons that preferentially jump to nearby sites to identify a new insertional mutation (see <http://www.arabidopsis.org/abrc/ima/jsp>). (b) You can construct a gene that has sense and antisense sequences transcribed to a single mRNA molecule (see Figure 16.23b), introduce it into *Arabidopsis* plants by *A. tumefaciens*-mediated transformation, and study its effect(s) on the expression of the gene and the phenotype of transgenic plants. The transcript will form a partially base-paired hairpin that will enter the RISC silencing pathway and block the expression of the gene (see Figure 16.23b).

**16.28** Let’s check the Salk Institute’s Genome Analysis Laboratory web site (<http://signal.salk.edu/cgi-bin/tdnaexpress>) to see if any of their T-DNA lines have insertions in the gene shown in the previous question. At the SIGnAL web site, scroll down to “Blast” and paste or type the sequence in the box. The resulting map will show the location of mapped T-DNA insertions relative to the location of the gene (green rectangle at the top). The blue arrows at the top right will let you focus on just the short region containing the gene or relatively long regions of chromosome 4 of *Arabidopsis*. Are there any T-DNA insertions in the gene in question? Near the gene?

**ANS:** A search of the Salk Institute’s Genome Analysis Laboratory (SIGnAL) web site reveals that there are numerous T-DNA insertions (Salk T-DNA insertions and several others) located in the promoter region of this gene. Moreover, there are two insertion lines (FLAG\_177C02 and FLAG\_216D01) in the collection at the Institute of Agronomic Research in Versailles, France, with inserts in the coding region of this gene. All of these insertion lines in these collections are available to researchers on request. Thus, the function of this gene can be studied by using these insertion lines.

**16.29** The CRISPR/Cas9 anti-phage immunity system in *Streptococcus pyogenes* deploys a variety of crRNAs derived from the spacer and repeat sequences in the CRISPR array in the *S. pyogenes* genome. In combination with the transactivating RNA (tracrRNA), these crRNAs guide the Cas9 endonuclease to complementary sequences in infecting phage genomes, whereupon Cas9 cleaves the phage DNA. A requirement for cleavage is that the targeted phage DNA sequence be immediately upstream of a protospacer adjacent motif (PAM), which in the *S. pyogenes* system is 5'-NGG-3'. Why is it important that the CRISPR array in the *S. pyogenes* genome not contain this PAM?



**ANS:** It is important that the CRISPR array in the *S. pyogenes* genome does not contain the PAM 5'NGG-3' because if it did, crRNAs generated from the array could target the Cas9 endonuclease to the array and cleave it, resulting in breakage of the *S. pyogenes* chromosome.

**16.30** The *Streptococcus pyogenes* Cas9 endonuclease can be targeted to a specific genomic DNA sequence by an sgRNA that at its 5' end has 20 nucleotides complementary to the target sequence. If this target sequence is immediately upstream of the protospacer adjacent motif (PAM) 5'NGG-3', Cas9 will cleave the target DNA. Suppose you have chosen a 20-nucleotide target sequence in the genome of *Drosophila melanogaster* and that this sequence is next to the required PAM. How could you determine if Cas9 will cleave only this sequence in the *Drosophila* genome?

**ANS:** You could use the 20-nucleotide sequence as a query in BLAST to scan the sequenced portion of the *Drosophila* genome to see if all or part of the target sequence is present anywhere else. If this sequence is present elsewhere, then you could check to see if the PAM 5'-NGG-3' is immediately downstream of the sequence. If it is, then the Cas9 endonuclease will cleave at this site as well as at the intended target site.

**16.31** How could the CRISPR/Cas9 system be used to create a translocation between two autosomes in cultured human cells?

**ANS:** Create two sgRNAs, one to target a sequence on a particular autosome and the other to target a sequence on a different autosome. Then introduce these sgRNAs and the Cas9 endonuclease into cultured cells to induce breakage at the two target sites. The broken DNA molecules may be repaired by the NHEJ pathway, and if they are, the broken pieces of different autosomes could be joined covalently, creating a reciprocal translocation.

## CHAPTER 17

**17.1** How can inducible and repressible enzymes of microorganisms be distinguished?

**ANS:** By studying the synthesis or lack of synthesis of the enzyme in cells grown on chemically defined media. If the enzyme is synthesized only in the presence of a certain metabolite or a particular set of metabolites, it is probably inducible. If it is synthesized in the absence but not in the presence of a particular metabolite or group of metabolites, it is probably repressible.

**17.2** Distinguish between (a) repression and (b) feedback inhibition caused by the end-product of a biosynthetic pathway. How do these two regulatory phenomena complement each other to provide for the efficient regulation of metabolism?

**ANS:** Repression occurs at the level of transcription during enzyme synthesis. The end-product, or a derivative of the end-product, of a repressible system acts as an

effector molecule that usually, if not always, combines with the product of one or more regulator genes to turn off the *synthesis* of the enzymes in the biosynthetic pathway. Feedback inhibition occurs at the level of enzyme activity; it usually involved the first enzyme of the biosynthetic pathway. Feedback inhibition thus brings about an immediate arrest of the biosynthesis of the end-product. All together, feedback inhibition and repression rapidly and efficiently turn off the synthesis of both the enzymes and the end-products that no longer need to be synthesized by the cell.

**17.3** In the lactose operon of *E. coli*, what is the function of each of the following genes or sites: (a) regulator, (b) operator, (c) promoter, (d) structural gene *Z*, and (e) structural gene *Y*?

**ANS: Gene or Regulatory**

Element	Function
(a) Regulator gene	Encodes the repressor
(b) Operator	Binding site of repressor
(c) Promoter	Binding site of RNA polymerase and CAP-cAMP complex
(d) Structural gene <i>Z</i>	Encodes $\beta$ -galactosidase
(e) Structural gene <i>Y</i>	Encodes $\beta$ -galactoside permease

**17.4** What would be the result of inactivation by mutation of the following genes or sites in the *E. coli* lactose operon: (a) regulator, (b) operator, (c) promoter, (d) structural gene *Z*, and (e) structural gene *Y*?

**ANS:** (a) Constitutive synthesis of the *lac* enzymes.  
 (b) Constitutive synthesis of the *lac* enzymes.  
 (c) Uninducibility of the *lac* enzymes.  
 (d) No  $\beta$ -galactosidase activity.  
 (e) No  $\beta$ -galactoside permease activity.

**17.5** Groups of alleles associated with the lactose operon are as follows (in order of dominance for each allelic series): repressor, *I*<sup>-</sup> (superrepressor), *I*<sup>+</sup> (inducible), and *I*<sup>-</sup> (constitutive); operator, *O*<sup>-</sup> (constitutive, *cis*<sup>-</sup> dominant) and *O*<sup>+</sup> (inducible, *cis*-dominant); structural, *Z*<sup>+</sup> and *Y*<sup>+</sup>. (a) Which of the following genotypes will produce  $\beta$ -galactosidase and  $\beta$ -galactoside permease if lactose is present: (1) *I*<sup>+</sup>*O*<sup>+</sup>*Z*<sup>+</sup>*Y*<sup>+</sup>, (2) *I*<sup>-</sup>*O*<sup>-</sup>*Z*<sup>+</sup>*Y*<sup>+</sup>, (3) *I*<sup>0</sup>*O*<sup>-</sup>*Z*<sup>+</sup>*Y*<sup>+</sup>, (4) *I*<sup>0</sup>*O*<sup>+</sup>*Z*<sup>+</sup>*Y*<sup>+</sup>, and (5) *I*<sup>-</sup>*O*<sup>+</sup>*Z*<sup>+</sup>*Y*<sup>+</sup>? (b) Which of the above genotypes will produce  $\beta$ -galactosidase and  $\beta$ -galactoside permease if lactose is absent? Why?

**ANS:** (a) 1, 2, 3, and 5. Genotype 1 is wild-type and inducible, whereas genotypes 2, 3, and 5 are constitutive; all except 4 will produce  $\beta$ -galactosidase and  $\beta$ -galactoside permease in the presence of lactose. However, genotype 4 has a superrepressor mutation (*I*<sup>-</sup>) and is uninducible with normal

levels of lactose. (b) 2, 3, and 5. In genotypes 2 and 3, the repressor cannot bind to  $O^c$  and in genotype 5, no repressor is made; both situations render the operon constitutive.

- 17.6** Assume that you have discovered a new strain of *E. coli* that has a mutation in the *lac* operator region that causes the wild-type repressor protein to bind irreversibly to the operator. You have named this operator mutant  $O^{sb}$  for “superbinding” operator. (a) What phenotype would a partial diploid of genotype  $I^+ O^{sb} Z^- Y^- / I^+ O^+ Z^+ Y^-$  have with respect to the synthesis of the enzymes  $\beta$ -galactosidase and  $\beta$ -galactoside permease? (b) Does your new  $O^{sb}$  mutation exhibit *cis* or *trans* dominance in its effects on the regulation of the *lac* operon?

**ANS:** (a)  $\beta$ -Galactosidase will be produced only when lactose is present. Permease will not be produced at all. (b) *cis* dominance.

- 17.7** Why is the  $O^c$  mutation in the *E. coli lac* operon epistatic to the  $I^f$  mutation?

**ANS:** The  $O^c$  mutant prevents the repressor from binding to the operator. The  $I^f$  mutant repressor cannot bind to  $O^c$ . The  $I^f$  mutant protein has a defect in the allosteric site that binds allolactose but has a normal operator binding site. Therefore, because the single  $O^c$  mutant would have the same phenotype as the  $O^c I^f$  double mutant, the  $O^c$  mutation is, by definition, epistatic to  $I^f$ .

- 17.8** For each of the following partial diploids indicate whether enzyme synthesis is constitutive or inducible (see Problem 17.5 for dominance relationships):

- (a)  $I^+ O^+ Z^+ Y^+ / I^+ O^+ Z^+ Y^+$ , (b)  $I^+ O^+ Z^+ Y^+ / I^+ O^c Z^+ Y^+$ ,  
 (c)  $I^+ O^c Z^+ Y^+ / I^+ O^c Z^+ Y^+$ , (d)  $I^+ O^+ Z^+ Y^+ / I^- O^+ Z^+ Y^+$ ,  
 (e)  $I^- O^+ Z^+ Y^+ / I^- O^+ Z^+ Y^+$ .

Why?

**ANS:** (a) Inducible, this is the wild-type genotype and phenotype.

(b) Constitutive, the  $O^c$  mutation produces an operator that is not recognized by the *lac* repressor.

(c) Constitutive, same as for (b).

(d) Inducible,  $I^+$  is dominant to  $I^-$ .

(e) Constitutive, no active repressor is synthesized in this bacterium.

- 17.9** Write the partial diploid genotype for a strain that will (a) produce  $\beta$ -galactosidase constitutively and permease inducibly and (b) produce  $\beta$ -galactosidase constitutively but not permease either constitutively or inducibly, even though a  $Y^+$  gene is known to be present.

**ANS:** (a)  $I^+ O^c Z^+ Y^- / I^+ O^+ Z^+ Y^+$  (b)  $I^+ O^c A^+ Y^- / I^+ O^+ Z^+ Y^+$

- 17.10** As a genetics historian, you are repeating some of the classic experiments conducted by Jacob and Monod with the lactose operon in *E. coli*. You use an F plasmid to

construct several *E. coli* strains that are partially diploid for the *lac* operon. You construct strains with the following genotypes: (1)  $I^+ O^c Z^+ Y^- / I^+ O^+ Z^- Y^+$ , (2)  $I^+ O^c Z^- Y^+ / I^+ O^+ Z^+ Y^-$ , (3)  $I^- O^+ Z^+ Y^+ / I^+ O^+ Z^- Y^-$ , (4)  $I^+ O^+ Z^- Y^- / I^+ O^+ Z^+ Y^+$ , and (5)  $I^+ O^c Z^+ Y^+ / I^+ O^+ Z^- Y^+$ . (a) Which of these strains will produce functional  $\beta$ -galactosidase in both the presence and absence of lactose? (b) Which of these strains will exhibit constitutive synthesis of functional  $\beta$ -galactoside permease? (c) Which of these strains will express both gene *Z* and gene *Y* constitutively and will produce functional products ( $\beta$ -galactosidase and  $\beta$ -galactoside permease) of both genes? (d) Which of these strains will show *cis* dominance of *lac* operon regulatory elements? (e) Which of these strains will exhibit *trans* dominance of *lac* operon regulatory elements?

**ANS:** (a) 1, 5.

(b) 2, 5.

(c) 5.

(d) 1, 2, 5.

(e) 3, 4.

- 17.11** Constitutive mutations produce elevated enzyme levels at all times; they may be of two types:  $O^c$  or  $I^-$ . Assume that all other DNA present is wild-type. Outline how the two constitutive mutants can be distinguished with respect to (a) map position, (b) regulation of enzyme levels in  $O^c/O^+$  versus  $I^-/I^+$  partial diploids, and (c) the position of the structural genes affected by an  $O^c$  mutation versus the genes affected by an  $I^-$  mutation in a partial diploid.

**ANS:** (a) The  $O^c$  mutations map very close to the *Z* structural gene;  $I^-$  mutations map slightly farther from the structural gene (but still very close by; see Figure 17.5).

(b) An  $I^+ O^+ Z^+ Y^+ / I^+ O^c Z^+ Y^+$  partial diploid would exhibit constitutive synthesis of  $\beta$ -galactosidase and  $\beta$ -galactoside permease, whereas an  $I^+ O^+ Z^+ Y^+ / I^- O^+ Z^+ Y^+$  partial diploid would be inducible for the synthesis of these enzymes.

(c) The  $O^c$  mutation is *cis*-dominant; the  $I^-$  mutation is *trans*-recessive.

- 17.12** How could the tryptophan operon in *E. coli* have developed and been maintained by evolution?

**ANS:** The system could have developed from a series of tandem duplications of a single ancestral gene. Mutational changes that make the system more efficient and, therefore, favored by natural selection could have brought the system to its present level of efficiency.

- 17.13** Of what biological significance is the phenomenon of catabolite repression?

**ANS:** Catabolite repression has apparently evolved to assure the use of glucose as a carbon source when this carbohydrate is available, rather than less efficient energy sources.

**17.14** How might the concentration of glucose in the medium in which an *E. coli* cell is growing regulate the intracellular level of cyclic AMP?

**ANS:** Possibly by directly or indirectly inhibiting the enzyme adenylylase, which catalyzes the synthesis of cyclic AMP from ATP.

**17.15** Is the CAP–cAMP effect on the transcription of the *lac* operon an example of positive or negative regulation? Why?

**ANS:** Positive regulation; the CAP–cAMP complex has a positive effect on the expression of the *lac* operon. It functions in turning on the transcription of the structural genes in the operon.

**17.16** Would it be possible to isolate *E. coli* mutants in which the transcription of the *lac* operon is not sensitive to catabolite repression? If so, in what genes might the mutations be located?

**ANS:** Yes; in the gene encoding CAP. Some mutations in this gene might result in a CAP that binds to the promoter in the absence of cAMP. Also, mutations in the gene (or genes) coding for the protein (or proteins) that regulate the cAMP level as a function of glucose concentration.

**17.17** Using examples, distinguish between negative regulatory mechanisms and positive regulatory mechanisms.

**ANS:** Negative regulatory mechanisms, such as that involving the repressor in the lactose operon, block the transcription of the structural genes of the operon, whereas positive mechanisms, such as the CAP–cAMP complex in the *lac* operon, promote the transcription of the structural genes of the operon.

**17.18** The following table gives the relative activities of the enzymes  $\beta$ -galactosidase and  $\beta$ -galactoside permease in cells with different genotypes at the *lac* locus in *E. coli*. The level of activity of each enzyme in wild-type *E. coli* not carrying F's was arbitrarily set at 100; all other values are relative to the observed levels of activity in these wild-type bacteria. Based on the data given in the table for genotypes 1 through 4, fill in the levels of enzyme activity that would be expected for the fifth genotype.

Genotype	$\beta$ -Galactoside		$\beta$ -Galactosidase Permease	
	– Inducer	+ Inducer	– Inducer	+ Inducer
1. $I^+O^+Z^+Y^+$	0.1	100	0.1	100
2. $I^-O^+Z^+Y^+$	100	100	100	100
3. $I^+O^-Z^+Y^+$	25	100	25	100
4. $I^-O^+Z^+Y^- / F' I^-O^+Z^+Y^+$	200	200	100	100
5. $I^-O^-Z^-Y^+ / F' I^+O^+Z^+Y^+$	_____	_____	_____	_____

**ANS:** 0.1; 100; 25.1; 200.

**17.19** The rate of transcription of the *trp* operon in *E. coli* is controlled by both (1) repression/derepression and (2) attenuation. By what mechanisms do these two regulatory processes modulate *trp* operon transcript levels?

**ANS:** Repression/derepression of the *trp* operon occurs at the level of transcription initiation, modulating the frequency at which RNA polymerase initiates transcription from the *trp* operon promoters. Attenuation modulates *trp* transcript levels by altering the frequency of termination of transcription within the *trp* operon leader region (*trpL*).

**17.20** What effect will deletion of the *trpL* region of the *trp* operon have on the rates of synthesis of the enzymes encoded by the five genes in the *trp* operon in *E. coli* cells growing in the presence of tryptophan?

**ANS:** Deletion of the *trpL* region would result in the levels of the tryptophan biosynthetic enzymes in cells growing in the presence of tryptophan being increased about 10-fold because attenuation would no longer occur if this region were absent.

**17.21** By what mechanism does the presence of tryptophan in the medium in which *E. coli* cells are growing result in premature termination or attenuation of transcription of the *trp* operon?

**ANS:** First, remember that transcription and translation are coupled in prokaryotes. When tryptophan is present in cells, tryptophan-charged tRNA<sup>Trp</sup> is produced. This allows translation of the *trp* leader sequence through the two UGG Trp codons to the *trp* leader sequence UGA termination codon. This translation of the *trp* leader region prevents base-pairing between the partially complementary mRNA leader sequences 75–83 and 110–121 (see Figure 17.15b), which in turn permits formation of the transcription–termination “hairpin” involving leader sequences 110–121 and 126–134 (see Figure 17.15c).

**17.22** Suppose that you used site-specific mutagenesis to modify the *trpL* sequence such that the two UGG Trp codons at positions 54–56 and 57–60 (see Figure 17.14) in the mRNA leader sequence were changed to GGG Gly codons. Will attenuation of the *trp* operon still be regulated by the presence or absence of tryptophan in the medium in which the *E. coli* cells are growing?

**ANS:** No. Attenuation of the *trp* operon would now be controlled by the presence or absence of Gly-tRNA<sup>Gly</sup>.

**17.23** What do *trp* attenuation and the lysine riboswitch have in common?

**ANS:** Both *trp* attenuation and the lysine riboswitch turn off gene expression by terminating transcription upstream from the coding regions of the regulated genes. Both involve the formation of alternative mRNA secondary structures—switching between the formation of antiterminator and transcription–terminator hairpins—in response to the presence or absence of a specific

metabolite (compare Figure 17.15 and Figure 2 in the Focus On The Lysine Riboswitch).

**17.24** Would attenuation of the type that regulates the level of *trp* transcripts in *E. coli* be likely to occur in eukaryotic organisms?

**ANS:** No. Since transcription (nucleus) and translation (cytoplasm) are not coupled in eukaryotes, attenuation of the type occurring in prokaryotes would not be possible.

## CHAPTER 18

**18.1** Operons are common in bacteria but not in eukaryotes. Suggest a reason why.

**ANS:** In multicellular eukaryotes, the environment of an individual cell is relatively stable. There is no need to respond quickly to changes in the external environment. In addition, the development of a multicellular organism involves complex regulatory hierarchies composed of hundreds of different genes. The expression of these genes is regulated spatially and temporally, often through intricate intercellular signaling processes.

**18.2** In bacteria, translation of an mRNA begins before the synthesis of that mRNA is completed. Why is this “coupling” of transcription and translation not possible in eukaryotes?

**ANS:** Coupling of transcription and translation is not possible in eukaryotes because these two processes take place in different cellular compartments—transcription in the nucleus and translation in the cytoplasm.

**18.3** Muscular dystrophy in humans is caused by mutations in an X-linked gene that encodes a protein called dystrophin. What techniques could you use to determine if this gene is active in different types of cells, say skin cells, nerve cells, and muscle cells?

**ANS:** Activity of the *dystrophin* gene could be assessed by blotting RNA extracted from the different types of cells and hybridizing it with a probe from the gene (northern blotting); or the RNA could be reverse transcribed into cDNA using one or more primers specific to the *dystrophin* gene and the resulting cDNA could be amplified by the polymerase chain reaction (RT-PCR). Another technique would be to hybridize *dystrophin* RNA *in situ*—that is, in the cells themselves—with a probe from the gene. It would also be possible to check each cell type for production of dystrophin protein by using anti-dystrophin antibodies to analyze proteins from the different cell types on western blots or analyze the proteins in the cells themselves—that is, *in situ*.

**18.4** Why do steroid hormones interact with receptors inside the cell, whereas peptide hormones interact with receptors on the cell surface?

**ANS:** Steroid hormones are small, lipid-soluble molecules that have little difficulty passing through the cell membrane.

Peptide hormones are typically too large to pass through the cell membrane freely; rather, their influence must be mediated by a signaling system that begins with a membrane-bound receptor protein that binds the hormone.

**18.5** In the polytene chromosomes of *Drosophila* larvae (Chapter 6), some bands form large “puffs” when the larvae are subjected to high temperatures. How could you show that these puffs contain genes that are vigorously transcribed in response to this heat shock treatment?

**ANS:** One procedure would be to provide larvae with radioactively labeled UTP, a building block of RNA, under different conditions—with and without heat shock. Then prepare samples of polytene cells from these larvae for autoradiography. If the heat shock-induced puffs contain genes that are vigorously transcribed, the radioactive signal should be abundant in the puffs.

**18.6** How would you distinguish between an enhancer and a promoter?

**ANS:** An enhancer can be located upstream, downstream, or within a gene, and it functions independently of its orientation. A promoter is almost always immediately upstream of a gene and it functions only in one direction with respect to the gene.

**18.7** Tropomyosins are proteins that mediate the interaction of actin and troponin, two proteins involved in muscle contractions. In higher animals, tropomyosins exist as a family of closely related proteins that share some amino acid sequences but differ in others. Explain how these proteins could be created from the transcript of a single gene.

**ANS:** By alternate splicing of the transcript.

**18.8** A polypeptide consists of three separate segments of amino acids, A—B—C. Another polypeptide contains segments A and C but not segment B. How might you determine if these two polypeptides are produced by translating alternately spliced versions of RNA from a single gene or by translating mRNA from two different genes?

**ANS:** Southern blotting of genomic DNA digested with an appropriate restriction enzyme, followed by hybridization of the blot with a probe containing the DNA encoding segments A and B, or B and C, or at least parts of these adjacent segments. If one DNA fragment is detected on the blot, the two polypeptides are encoded by a single gene whose RNA is alternately spliced to produce two mRNAs. If two DNA fragments are detected, the two polypeptides are encoded by two different genes.

**18.9** What techniques could be used to show that a plant gene is transcribed when the plant is illuminated with light?

**ANS:** Northern blotting of RNA extracted from plants grown with and without light, or PCR amplification of cDNA made by reverse transcribing these same RNA extracts.

**18.10** When introns were first discovered, they were thought to be genetic “junk”—that is, sequences without any useful function. In fact, they appeared to be worse than junk because they actually interrupted the coding sequences of genes. However, among eukaryotes, introns are pervasive and anything that is pervasive in biology usually has a function. What function might introns have? What benefit might they confer on an organism?

**ANS:** Introns make it possible for genes to encode different—but related—polypeptides by alternate splicing of their RNA transcripts.

**18.11** The GAL4 transcription factor in yeast regulates two adjacent genes, *GAL1* and *GAL10*, by binding to DNA sequences between them. These two genes are transcribed in opposite directions on the chromosome, one to the left of the GAL4 protein’s binding site and the other to the right of this site. What property of enhancers does this situation illustrate?

**ANS:** That enhancers can function in either orientation.

**18.12** Using the techniques of genetic engineering, a researcher has constructed a fusion gene containing the heat-shock response elements from a *Drosophila hsp70* gene and the coding region of a jellyfish gene (*gfp*) for green fluorescent protein. This fusion gene has been inserted into the chromosomes of living *Drosophila* by the technique of transposon-mediated transformation (Chapter 21 on the Instructor Companion site). Under what conditions will the green fluorescent protein be synthesized in these genetically transformed flies? Explain.

**ANS:** The green fluorescent protein will be made after the flies are heat shocked.

**18.13** Suppose that the segment of the *hsp70* gene that was used to make the *hsp70/gfp* fusion in the preceding problem had mutations in each of its heat-shock response elements. Would the green fluorescent protein encoded by this fusion gene be synthesized in genetically transformed flies?

**ANS:** Probably not unless the promoter of the *gfp* gene is recognized and transcribed by the *Drosophila* RNA polymerase independently of the heat-shock response elements.

**18.14** The polypeptide products of two different genes, *A* and *B*, each function as transcription factors. These polypeptides interact to form dimers: AA homodimers, BB homodimers, and AB heterodimers. If the *A* and *B* polypeptides are equally abundant in cells, and if dimer formation is random, what is the expected ratio of homodimers to heterodimers in these cells?

**ANS:** With equal abundance of the *A* and *B* polypeptides, AA homodimers should constitute 1/4 of the total dimers formed, BB homodimers should constitute 1/4 of the total, and AB heterodimers should constitute 1/2 of the total. The expected ratio of homodimers to heterodimers is therefore  $(1/4 + 1/4):(1/2) = 1:1$ .

**18.15** A particular transcription factor binds to enhancers in 40 different genes. Predict the phenotype of individuals homozygous for a frameshift mutation in the coding sequence of the gene that specifies this transcription factor.

**ANS:** The mutation is likely to be lethal in homozygous condition because the transcription factor controls so many different genes, and a frameshift mutation in the coding sequence will almost certainly destroy the transcription factor’s function.

**18.16** The alternately spliced forms of the RNA from the *Drosophila doublesex* gene encode proteins that are needed to block the development of one or the other set of sexual characteristics. The protein that is made in female animals blocks the development of male characteristics, and the protein that is made in male animals blocks the development of female characteristics. Predict the phenotype of XX and XY animals homozygous for a null mutation in the *doublesex* gene.

**ANS:** Both XX and XY animals would develop as intersexes because neither of the forms of the doublesex protein will be able to block sexual development. In these animals, both developmental pathways will be carried out, leading to tissues that have both male and female characteristics.

**18.17** The RNA from the *Drosophila Sex-lethal (Sxl)* gene is alternately spliced. In males, the sequence of the mRNA derived from the primary transcript contains all eight exons of the *Sxl* gene. In females, the mRNA contains only seven of the exons because during splicing exon 3 is removed from the primary transcript along with its flanking introns. The coding region in the female’s mRNA is therefore shorter than it is in the male’s mRNA. However, the protein encoded by the female’s mRNA is longer than the one encoded by the male’s mRNA. How might you explain this paradox?

**ANS:** Exon 3 contains an in-frame stop codon. Thus, the protein translated from the *Sxl* mRNA in males will be shorter than the protein translated from the shorter *Sxl* mRNA in females.

**18.18** In *Drosophila*, expression of the *yellow* gene is needed for the formation of dark pigment in many different tissues; without this expression, a tissue appears yellow in color. In the wings, the expression of the *yellow* gene is controlled by an enhancer located upstream of the gene’s transcription initiation site. In the tarsal claws, expression is controlled by an enhancer located within the gene’s only intron. Suppose that by genetic engineering, the wing enhancer is placed within the intron and the claw enhancer is placed upstream of the transcription initiation site. Would a fly that carried this modified *yellow* gene in place of its natural *yellow* gene have darkly pigmented wings and claws? Explain.

**ANS:** Yes. Enhancers are able to function in different positions in and around a gene.

**18.19** A researcher suspects that a 550-bp-long intron contains an enhancer that drives expression of an *Arabidopsis* gene specifically in root tip tissue. Outline an experiment to test this hypothesis.

**ANS:** The intron could be placed in a GUS expression vector, which could then be inserted into *Arabidopsis* plants. If the intron contains an enhancer that drives gene expression in root tips, transgenic plants should show GUS expression in their root tips. See the Problem-Solving Skills feature in Chapter 18 for an example of this type of analysis.

**18.20** What is the nature of each of the following classes of enzymes? What does each type of enzyme do to chromatin? (a) HATs, (b) HDACs, (c) HMTs.

**ANS:** (a) HATs, histone acetyl transferases, transfer acetyl groups to the amino acid lysine in histones; (b) HDACs, histone deacetylases, remove acetyl groups from these lysines; (c) HMTs, histone methyl transferases, transfer methyl groups to lysine, arginine, and histidine in histones.

**18.21** In *Drosophila* larvae, the single X chromosome in males appears diffuse and bloated in the polytene cells of the salivary gland. Is this observation compatible with the idea that X-linked genes are hyperactivated in *Drosophila* males?

**ANS:** Yes. The diffuse, bloated appearance indicates that the genes on this chromosome are being transcribed vigorously—the chromatin is “open for business.”

**18.22** Suppose that the LCR of the  $\beta$ -globin gene cluster was deleted from one of the two chromosomes 11 in a man. What disease might this deletion cause?

**ANS:** The LCR regulates the expression of all the genes linked to it. Deletion of the LCR would ablate or impair globin gene expression from one of the two chromosomes 11. With less  $\beta$ -globin being produced, the individual would likely suffer from anemia.

**18.23** Would double-stranded RNA derived from an intron be able to induce RNA interference?

**ANS:** Short interfering RNAs target messenger RNA molecules, which are devoid of introns. Thus, if siRNA were made from double-stranded RNA derived from an intron, it would be ineffective against an mRNA target.

**18.24** An RNA interference-like phenomenon has been implicated in the regulation of transposable elements. In *Drosophila*, two of the key proteins involved in this regulation are encoded by the genes *aubergine* and *piwi*. Flies that are homozygous for mutant alleles of these genes are lethal or sterile, but flies that are heterozygous for them are viable and fertile. Suppose that you have strains of *Drosophila* that are heterozygous for *aubergine* or *piwi* mutant alleles. Why might the genomic mutation rate in these mutant strains be greater than the genomic mutation rate in a wild-type strain?

**ANS:** The *aubergine* and *piwi* gene products mediate the RNAi-like response. Reduction in the amount of aubergine or piwi protein would likely impair the organism's ability to mount this response, and without a vigorous capacity for regulation, transposable elements would be more likely to move in the genome. This movement would likely cause mutations because the transposons could insert into genes and inactivate them. Thus, *Drosophila* that are heterozygous for mutations in *aubergine* or *piwi* might experience higher mutation rates than *Drosophila* lacking these mutations.

**18.25** Suppose that female mice homozygous for the *a* allele of the *Igf2* gene are crossed to male mice homozygous for the *b* allele of this gene. Which of these two alleles will be expressed in the F<sub>1</sub> progeny?

**ANS:** The paternally contributed allele (*b*) will be expressed in the F<sub>1</sub> progeny.

**18.26** Epigenetic states are transmitted clonally through cell division. What kinds of observations indicate that these states can be reversed or reset?

**ANS:** Here are two observations that show reversal or resetting of an epigenetic state: (1) For imprinted genes in mammals, the epigenetic state can be reset when the gene passes through the germ line of the opposite sex. (2) Genes on the inactive X chromosome in mammals are reactivated in the female germ line.

**18.27** A researcher hypothesizes that in mice gene *A* is actively transcribed in liver cells, whereas gene *B* is actively transcribed in brain cells. Describe procedures that would allow the researcher to test this hypothesis.

**ANS:** RNA could be isolated from liver and brain tissue. Northern blotting or RT-PCR with this RNA could then establish which of the genes (*A* or *B*) is transcribed in which tissue. For northern blotting, the RNA samples would be fractionated in a denaturing gel and blotted to a membrane and then the RNA on the membrane would be hybridized with gene-specific probes, first for one gene, then for the other (or the researcher could prepare two separate blots and hybridize each one with a different probe). For RT-PCR, the RNA samples would be reverse transcribed into cDNA using primers specific for each gene; then the cDNA molecules would be amplified by standard PCR and the products of the amplifications would be fractionated by gel electrophoresis to determine which gene's RNA was present in the original samples.

**18.28** Suppose that the hypothesis mentioned in the previous question is correct and that gene *A* is actively transcribed in liver cells, whereas gene *B* is actively transcribed in brain cells. The researcher now extracts equivalent amounts of chromatin from liver and brain tissues and treats these extracts separately with DNase I for a limited period of time. If the DNA that remains after the treatments is then fractionated by gel electrophoresis,

transferred to a membrane by Southern blotting, and hybridized with a radioactively labeled probe specific for gene *A*, which sample (liver or brain) will be expected to show the greater signal on the autoradiogram? Explain your answer.

**ANS:** The sample of chromatin from brain tissue would be expected to show the greater signal on a Southern blot hybridized with a probe specific for gene *A*. The reason is that this gene is not so well transcribed in brain cells; consequently, it will be more resistant to digestion with DNase I in chromatin derived from brain cells than in chromatin derived from liver cells in which it is actively transcribed (and therefore more open to digestion with DNase I).

**18.29** Why do null mutations in the *msl* gene in *Drosophila* have no effect in females?

**ANS:** The *msl* gene is not functional in females.

**18.30** Suppose that a woman carries an X chromosome in which the *XIST* locus has been deleted. The woman's other X chromosome has an intact *XIST* locus. What pattern of X-inactivation would be observed throughout the woman's body?

**ANS:** The X chromosome containing the intact *XIST* locus will be silenced in all cases because that locus is located with the X inactivation center (XIC) and aids in silencing the inactive X.

**18.31** In *Drosophila*, the variegated phenotype of the *white mottled* allele is suppressed by a dominant autosomal mutation that knocks out the function of the gene for heterochromatin protein 1 (HP1), an important factor in heterochromatin formation. Flies with the *white mottled* allele and the suppressor mutation have an almost uniform red color in their eyes; without the suppressor mutation, the eyes are mosaics of red and white tissue. Can you suggest an explanation for the effect of the suppressor mutation?

**ANS:** HP1, the protein encoded by the wild-type allele of the suppressor gene, is involved in chromatin organization. Perhaps this heterochromatic protein spreads from the region near the inversion breakpoint in the chromosome that carries the *white mottled* allele and brings about the "heterochromatization" of the *white* locus. When HP1 is depleted by knocking out one copy of the gene encoding it—that is, by putting the suppressor mutation into the fly's genotype, the "heterochromatization" of the *white* locus would be less likely to occur, and perhaps not occur at all. The *white* locus would then function fully in all eye cells, producing a uniform red eye color.

**18.32** The sheep Dolly (Chapter 2) was the first cloned mammal. Dolly was created by implanting a nucleus from a cell taken from the udder of a female sheep into an enucleated egg. This nucleus had two X chromosomes, and because it came from a differentiated cell, one of them must have been inactivated. If the udder cell was

heterozygous for at least one X-linked gene whose expression you could assay, how could you determine if all of Dolly's cells had the same X chromosome inactivated? If, upon testing, Dolly's cells prove to be mosaic for X chromosome activity—that is, different X's are active in different clones of cells—what must have happened during her embryological development?

**ANS:** Take samples of cells from Dolly and determine if the products of both alleles of the X-linked gene are present in them. If the products of both alleles are present, then Dolly must be a genetic mosaic for X chromosome activity. Thus, during her development, the pattern of X-inactivation that existed in the udder cell from which she was derived must have been reset. If only one of the gene's products is detected—and if the sample of cells is representative of all Dolly's cells—then Dolly must have maintained the pattern of X-inactivation that existed in the udder cell from which she was derived.

## CHAPTER 19

**19.1** If heart disease is considered to be a threshold trait, what genetic and environmental factors might contribute to the underlying liability for a person to develop this disease?

**ANS:** Some of the genes implicated in heart disease are listed in Table 19.2. Environmental factors might include diet, amount of exercise, and whether or not the person smokes.

**19.2** A wheat variety with red kernels (genotype  $A'A' B'B'$ ) was crossed with a variety with white kernels (genotype  $AA BB$ ). The  $F_1$  were intercrossed to produce an  $F_2$ . If each primed allele increases the amount of pigment in the kernel by an equal amount, what phenotypes will be expected in the  $F_2$ ? Assuming that the *A* and *B* loci assort independently, what will the phenotypic frequencies be?

**ANS:** 1/16 red; 4/16 = 1/4 dark pink; 6/16 pink; 4/16 = 1/4 light pink; 1/16 white.

**19.3** For alcoholism, the concordance rate for monozygotic twins is 55 percent, whereas for dizygotic twins, it is 28 percent. Do these data suggest that alcoholism has a genetic basis?

**ANS:** The concordance for monozygotic twins is almost twice as great as that for dizygotic twins. Monozygotic twins share twice as many genes as dizygotic twins. The data strongly suggest that alcoholism has a genetic basis.

**19.4** The height of the seed head in wheat at maturity is determined by several genes. In one variety, the head is just 9 inches above the ground; in another, it is 33 inches above the ground. Plants from the 9-inch variety were crossed to plants from the 33-inch variety. Among the  $F_1$ , the seed head was 21 inches above the ground. After self-fertilization, the  $F_1$  plants produced an  $F_2$  population in which 9-inch and 33-inch plants each appeared with a frequency of 1/256. (a) How many genes are involved in

the determination of seed head height in these strains of wheat? (b) How much does each allele of these genes contribute to seed head height? (c) If a 21-inch  $F_1$  plant were crossed to a 9-inch plant, how often would you expect 18-inch wheat to occur in the progeny?

**ANS:** (a) 4; (b) 3 inches; (c) frequency of 1/4.

**19.5** Assume that size in rabbits is determined by genes with equal and additive effects. From a total of 2012  $F_2$  progeny from crosses between true-breeding large and small varieties, eight rabbits were as small as the small variety and eight were as large as the large variety. How many size-determining genes were segregating in these crosses?

**ANS:** Because  $8/2012$  is approximately  $1/256 = (1/4)^4$ , it appears that four size-determining genes were segregating in the crosses.

**19.6** A sample of 20 plants from a population was measured in inches as follows: 18, 21, 20, 23, 20, 21, 20, 22, 19, 20, 17, 21, 20, 22, 20, 21, 20, 22, 19, and 23. Calculate (a) the mean, (b) the variance, and (c) the standard deviation.

**ANS:** (a) The mean is 20.45 inches. (b) The variance is 2.37 inches<sup>2</sup>. (c) The standard deviation is 1.54 inches.

**19.7** Quantitative geneticists use the variance as a measure of scatter in a sample of data; they calculate this statistic by averaging the squared deviations between each measurement and the sample mean. Why don't they simply measure the scatter by computing the average of the deviations without bothering to square them?

**ANS:** Because  $\Sigma(X_i - \text{mean}) = 0$ .

**19.8** Two inbred strains of corn were crossed to produce an  $F_1$ , which was then intercrossed to produce an  $F_2$ . Data on ear length from a sample of  $F_1$  and  $F_2$  individuals gave phenotypic variances of 15.2 cm<sup>2</sup> and 27.6 cm<sup>2</sup>, respectively. Why was the phenotypic variance greater for the  $F_2$  than for the  $F_1$ ?

**ANS:** For the  $F_1$ ,  $V_g = 0$  because they are all genetically identical and heterozygous; for the  $F_2$ ,  $V_g > 0$  because genetic differences result from the segregation and independent assortment of genes. Thus, in the  $F_2$ , the phenotypic variance has a pronounced genetic component.

**19.9** A study of quantitative variation for abdominal bristle number in female *Drosophila* yielded estimates of  $V_T = 6.08$ ,  $V_g = 3.17$ , and  $V_e = 2.91$ . What was the broad-sense heritability?

**ANS:**  $3.17/6.08 = 0.52$

**19.10** A researcher has been studying kernel number on ears of corn. In one highly inbred strain, the variance for kernel number is 426. Within this strain, what is the broad-sense heritability for kernel number?

**ANS:** The broad-sense heritability within a highly inbred strain is expected to be zero because there is no genetic variability.

**19.11** Measurements on ear length were obtained from three populations of corn—two inbred varieties and a randomly pollinated population derived from a cross between the two inbred strains. The phenotypic variances were 9.2 cm<sup>2</sup> and 9.6 cm<sup>2</sup> for the two inbred varieties and 26.4 cm<sup>2</sup> for the randomly pollinated population. Estimate the broad-sense heritability of ear length for these populations.

**ANS:**  $V_e$  is estimated by the average of the variances of the inbreds: 9.4 cm<sup>2</sup>.  $V_g$  is estimated by the difference between the variances of the randomly pollinated population and the inbreds:  $(26.4 - 9.4) = 17.0$  cm<sup>2</sup>. The broad-sense heritability is  $H^2 = V_g/V_T = 17.0/26.4 = 0.64$ .

**19.12** Figure 19.4 summarizes data on maturation time in populations of wheat. Do these data provide any insight as to whether or not this trait is influenced by dominance? Explain.

**ANS:** Because the  $F_1$  plants have maturation times midway between those of the parental strains, there seems to be little or no dominance for this trait.

**19.13** A quantitative geneticist claims that the narrow-sense heritability for body mass in human beings is 0.7, while the broad-sense heritability is only 0.3. Why must there be an error?

**ANS:** Broad-sense heritability must be greater than narrow-sense heritability because  $H^2 = V_g/V_T > V_a/V_T = h^2$ .

**19.14** The mean value of a trait is 100 units, and the narrow-sense heritability is 0.4. A male and a female measuring 124 and 126 units, respectively, mate and produce a large number of offspring, which are reared in an average environment. What is the expected value of the trait among these offspring?

**ANS:**  $(125 - 100)(0.4) + 100 = 110$  units.

**19.15** The narrow-sense heritability for abdominal bristle number in a population of *Drosophila* is 0.3. The mean bristle number is 12. A male with 10 bristles is mated to a female with 20 bristles, and a large number of progeny are scored for bristle number. What is the expected number of bristles among these progeny?

**ANS:**  $(15 - 12)(0.3) + 12 = 12.9$  bristles.

**19.16** A breeder is trying to decrease the maturation time in a population of sunflowers. In this population, the mean time to flowering is 100 days. Plants with a mean flowering time of only 90 days were used to produce the next generation. If the narrow-sense heritability for flowering time is 0.2, what will the average time to flowering be in the next generation?

**ANS:**  $(90 - 100)(0.2) + 100 = 98$  days.

**19.17** A fish breeder wishes to increase the rate of growth in a stock by selecting for increased length at 6 weeks after hatching. The mean length of 6-week-old fingerlings is currently 10 cm. Adult fish that had a mean length of



15 cm at 6 weeks of age were used to produce a new generation of fingerlings. Among these, the mean length was 12.5 cm. Estimate the narrow-sense heritability of fingerling length at 6 weeks of age and advise the breeder about the feasibility of the plan to increase growth rate.

**ANS:**  $b^2 = R/S = (12.5 - 10)/(15 - 10) = 0.5$ ; selection for increased growth rate should be effective.

**19.18** Leo's IQ is 86 and Julie's IQ is 110. The mean IQ in the population is 100. Assume that the narrow-sense heritability for IQ is 0.4. What is the expected IQ of Leo and Julie's first child?

**ANS:**  $(98 - 100)(0.4) + 100 = 99.2$ .

**19.19** One way to estimate a maximum value for the narrow-sense heritability is to calculate the correlation between half-siblings that have been reared apart and divide it by the fraction of genes that half-siblings share by virtue of common ancestry. A study of human half-siblings found that the correlation coefficient for height was 0.14. From this result, what is the maximum value of the narrow-sense heritability for height in this population?

**ANS:** Half-siblings share 25 percent of their genes. The maximum value for  $b^2$  is therefore  $0.14/0.25 = 0.56$ .

**19.20** A selection differential of 40  $\mu\text{g}$  per generation was used in an experiment to select for increased pupa weight in *Tribolium*. The narrow-sense heritability for pupa weight was estimated to be 0.3. If the mean pupa weight was initially 2000  $\mu\text{g}$  and selection was practiced for 10 generations, what was the mean pupa weight expected to become?

**ANS:** The response to selection in one generation is  $R = b^2 S = (0.3)(40 \mu\text{g}) = 12 \mu\text{g}$ . The cumulative effect over 10 generations is therefore  $10 \times 12 \mu\text{g} = 120 \mu\text{g}$ . Thus, the mean pupa weight should become 2120  $\mu\text{g}$ .

**19.21** On the basis of the observed correlations for personality traits shown in Table 19.5, what can you say about the value of the environmentality ( $C^2$  in Table 19.3)?

**ANS:** The correlations for MZT are not much different from those for MZA. Evidently, for these personality traits, the environmentality ( $C^2$  in Table 19.3) is negligible.

**19.22** Correlations between relatives provide estimates of the broad and narrow-sense heritabilities on the assumption that the genetic and environmental factors influencing quantitative traits are independent of each other and that they do not interact in some peculiar way. In Chapter 18, we considered epigenetic modifications of chromatin that regulate genes and noted the possibility that some of these modifications might be induced by environmental factors. How could epigenetic influences on complex traits be incorporated into the basic theory of quantitative genetics?

**ANS:** We might represent the value of a quantitative trait,  $T$ , as  $\mu + g + e + eg$ , where  $\mu$  is the mean of the population,  $g$  is the deviation due to genetic factors,  $e$  is the deviation due

to environmental factors, and  $eg$  is the deviation due to epigenetic factors arising from the interaction of genetic and environmental factors.

## CHAPTER 20

**20.1** The following data for the M–N blood types were obtained from native villages in Central and North America:

Group	Sample Size	M	MN	N
Central American	86	53	29	4
North American	278	78	61	139

Calculate the frequencies of the  $L^M$  and  $L^N$  alleles for the two groups.

**ANS:** Frequency of  $L^M$  in Central American population:  $p = (2 \times 53 + 29)/(2 \times 86) = 0.78$ ;  $q = 0.22$ . Frequency of  $L^M$  in North American population:  $p = (2 \times 78 + 61)/(2 \times 278) = 0.39$ ;  $q = 0.61$ .

**20.2** The frequency of an allele in a large randomly mating population is 0.2. What is the frequency of heterozygous carriers?

**ANS:**  $2pq = 2(0.2)(0.8) = 0.32$ .

**20.3** The incidence of recessive albinism is 0.0004 in a human population. If mating for this trait is random in the population, what is the frequency of the recessive allele?

**ANS:**  $q^2 = 0.0004$ ;  $q = 0.02$ .

**20.4** In a sample from an African population, the frequencies of the  $LM$  and  $LN$  alleles were 0.78 and 0.22, respectively. If the population mates randomly with respect to the M–N blood types, what are the expected frequencies of the M, MN, and N phenotypes?

**ANS:** **Phenotype**      **Hardy–Weinberg Frequency**

M                       $(0.78)^2 = 0.61$

MN                     $2(0.78)(0.22) = 0.34$

N                       $(0.22)^2 = 0.05$

**20.5** Human beings carrying the dominant allele  $T$  can taste the substance phenylthiocarbamide (PTC). In a population in which the frequency of this allele is 0.4, what is the probability that a particular taster is homozygous?

**ANS:** Frequency of tasters (genotypes  $TT$  and  $Tt$ ):  $(0.4)^2 + 2(0.4)(0.6) = 0.64$ . Frequency of  $TT$  tasters among all tasters:  $(0.4)^2/(0.64) = 0.25$ .

**20.6** A gene has three alleles,  $A_1$ ,  $A_2$ , and  $A_3$ , with frequencies 0.6, 0.3, and 0.1, respectively. If mating is random, predict the combined frequency of all the heterozygotes in the population.

**ANS:** Frequency of heterozygotes combined =  $2[(0.6)(0.3) + (0.3)(0.1) + ((0.6)(0.1))] = 0.54$ .

**20.7** Hemophilia is caused by an X-linked recessive allele. In a particular population, the frequency of males with hemophilia is  $1/4000$ . What is the expected frequency of females with hemophilia?

**ANS:**  $(0.00025)^2 = 6.25 \times 10^{-8}$ .

**20.8** In *Drosophila*, the ruby eye phenotype is caused by a recessive, X-linked mutant allele. The wild-type eye color is red. A laboratory population of *Drosophila* is started with 25 percent ruby-eyed females, 25 percent homozygous red-eyed females, 5 percent ruby-eyed males, and 45 percent red-eyed males. (a) If this population mates randomly for one generation, what is the expected frequency of ruby-eyed males and females? (b) What is the frequency of the recessive allele in each of the sexes?

**ANS:** (a) Half the males will be ruby-eyed and 5 percent ( $0.50 \times 0.10 \times 100$  percent) of the females will be ruby-eyed. (b) Among males, the frequency of the recessive allele will be 0.5, which was its frequency among females in the previous generation; among females, the frequency of the recessive allele will be  $(0.5 + 0.1)/2 = 0.3$ , which is the average of the frequencies of this allele in males and females in the previous generation.

**20.9** A trait determined by an X-linked dominant allele shows 100 percent penetrance and is expressed in 36 percent of the females in a population. Assuming that the population is in Hardy–Weinberg equilibrium, what proportion of the males in this population express the trait?

**ANS:** In females, the frequency of the dominant phenotype is 0.36. The frequency of the recessive phenotype is  $0.64 = q^2$ ; thus,  $q = 0.8$  and  $p = 0.2$ . The frequency of the dominant phenotype in males is therefore  $p = 0.2$ .

**20.10** A phenotypically normal couple has had one normal child and a child with cystic fibrosis, an autosomal recessive disease. The incidence of cystic fibrosis in the population from which this couple came is  $1/500$ . If their normal child eventually marries a phenotypically normal person from the same population, what is the risk that the newlyweds will produce a child with cystic fibrosis?

**ANS:** The probability that the unaffected child of the couple is a carrier of the mutant allele for cystic fibrosis is  $2/3$ . The probability that the mate of this individual is a carrier can be determined by using the population incidence of the disease. The mutant allele frequency is the square root of the incidence—0.045—and the frequency of heterozygotes under the assumption of random mating is  $2 \times 0.045 \times (1 - 0.045) = 0.086$ . If both individuals are carriers, the chance that they will have an affected child is  $1/4$ . Putting all this analysis together, the risk for the child to have cystic fibrosis is therefore  $2/3 \times 0.086 \times 1/4 = 0.014$ , which is seven times the incidence in the population at large.

**20.11** What frequencies of alleles  $A$  and  $a$  in a randomly mating population maximize the frequency of heterozygotes?

**ANS:** Frequency of heterozygotes  $= H = 2pq = 2p(1 - p)$ . Using calculus, take the derivative of  $H$  and set the result to zero to solve for the value of  $p$  that maximizes  $H$ :  $dH/dp = 2 - 4p = 0$  implies that  $p = 2/4 = 0.5$ .

**20.12** In an isolated population, the frequencies of the  $I^A$ ,  $I^B$ , and  $i$  alleles of the A–B–O blood type gene are, respectively, 0.15, 0.25, and 0.60. If the genotypes of the A–B–O blood type gene are in Hardy–Weinberg proportions, what fraction of the people who have type A blood in this population is expected to be homozygous for the  $I^A$  allele?

**ANS:** In a Hardy–Weinberg population, the frequency of  $I^A I^A$  homozygotes is  $(0.15)^2 = 0.0335$  and the frequency of  $I^A i$  heterozygotes is  $2 \times 0.15 \times 0.60 = 0.18$ . The sum of these frequencies—0.2025—is the frequency of individuals with type A blood. Thus, the frequency of  $I^A I^A$  homozygotes among all individuals with type A blood is  $0.0335/0.2025 = 0.1111$ .

**20.13** In a survey of moths collected from a natural population, a researcher found 51 dark specimens and 49 light specimens. The dark moths carry a dominant allele, and the light moths are homozygous for a recessive allele. If the population is in Hardy–Weinberg equilibrium, what is the estimated frequency of the recessive allele in the population? How many of the dark moths in the sample are likely to be homozygous for the dominant allele?

**ANS:** Under the assumption that the population is in Hardy–Weinberg equilibrium, the frequency of the allele for light coloration is the square root of the frequency of recessive homozygotes. Thus,  $q = \sqrt{0.49} = 0.7$ , and the frequency of the allele for dark color is  $1 - q = p = 0.3$ . From  $p^2 = 0.09$ , we estimate that  $0.09 \times 100 = 9$  of the dark moths in the sample are homozygous for the dominant allele.

**20.14** A population of Hawaiian *Drosophila* is segregating two alleles,  $P^1$  and  $P^2$ , of the phosphoglucose isomerase (*PGI*) gene. In a sample of 100 flies from this population, 30 were  $P^1 P^1$  homozygotes, 60 were  $P^1 P^2$  heterozygotes, and 10 were  $P^2 P^2$  homozygotes. (a) What are the frequencies of the  $P^1$  and  $P^2$  alleles in this sample? (b) Perform a chi-square test to determine if the genotypes in the sample are in Hardy–Weinberg proportions. (c) Assuming that the sample is representative of the population, how many generations of random mating would be required to establish Hardy–Weinberg proportions in the population?

**ANS:** (a)  $P^1 = 0.6$  and  $P^2 = 0.4$ . (b) Predict H–W proportions by multiplying the expected genotype frequencies by the sample size and compare these values with the observed genotype frequencies using a chi-square test:

Genotype	H-W Frequency	Predicted Number
$P^1P^1$	$0.6^2 = 0.36$	$0.36 \times 100 = 36$
$P^1P^2$	$2(0.6)(0.4) = 0.48$	$0.28 \times 100 = 48$
$P^2P^2$	$0.4^2 = 0.16$	$0.16 \times 100 = 16$

$\chi^2 = (30 - 36)^2/36 + (60 - 48)^2/48 + (10 - 16)^2/16 = 6.25$ ,  
 $df = 1$ ; 6.25 is greater than 3.841; therefore, the observed  
 genotypes are not in agreement with H-W.

- 20.15** In a large population that reproduces by random mating, the frequencies of the genotypes  $GG$ ,  $Gg$ , and  $gg$  are 0.04, 0.32, and 0.64, respectively. Assume that a change in the climate induces the population to reproduce exclusively by self-fertilization. Predict the frequencies of the genotypes in this population after many generations of self-fertilization.

**ANS:** Ultimate frequency of  $GG$  is 0.2; ultimate frequency of  $gg$  is 0.8.

- 20.16** The frequencies of the alleles  $A$  and  $a$  are 0.6 and 0.4, respectively, in a particular plant population. After many generations of random mating, the population goes through one cycle of self-fertilization. What is the expected frequency of heterozygotes in the progeny of the self-fertilized plants?

**ANS:**  $2pq(1 - F) = 2(0.6)(0.4)(1 - 0.5) = 0.24$ .

- 20.17** Each of two isolated populations is in Hardy-Weinberg equilibrium with the genotype frequencies shown below:

Genotype:	$AA$	$Aa$	$Aa$
Frequency in Population 1:	0.04	0.32	0.64
Frequency in Population 2:	0.64	0.32	0.04

(a) If the populations are equal in size and they merge to form a single large population, predict the allele and genotype frequencies in the large population immediately after merger.

(b) If the merged population reproduces by random mating, predict the genotype frequencies in the next generation.

(c) If the merged population continues to reproduce by random mating, will these genotype frequencies remain constant?

**ANS:** (a) Frequency of  $A$  in merged population is 0.5 and that of  $a$  is also 0.5; (b) 0.25 ( $AA$ ), 0.50 ( $Aa$ ), and 0.25 ( $aa$ ); (c) frequencies in (b) will persist.

- 20.18** A population consists of 25 percent tall individuals (genotype  $TT$ ), 25 percent short individuals (genotype  $tt$ ), and 50 percent individuals of intermediate height

(genotype  $Tt$ ). Predict the ultimate phenotypic and genotypic composition of the population if, generation after generation, mating is strictly assortative (i.e., tall individuals mate with tall individuals, short individuals mate with short individuals, and intermediate individuals mate with intermediate individuals).

**ANS:** Ultimately, all members of the population will either be  $TT$  or  $tt$ , each 50% of the total.

- 20.19** In controlled experiments with different genotypes of an insect, a researcher has measured the probability of survival from fertilized eggs to mature, breeding adults. The survival probabilities of the three genotypes tested are 0.92 (for  $GG$ ), 0.90 (for  $Gg$ ), and 0.56 (for  $gg$ ). If all breeding adults are equally fertile, what are the relative fitnesses of the three genotypes? What are the selection coefficients for the two least fit genotypes?

**ANS:** The relative fitnesses can be obtained by dividing each of the survival probabilities by the largest probability (0.92). Thus, the relative fitnesses are 1 for  $GG$ ,  $0.98 = 1 - 0.02$  for  $Gg$ , and  $0.61 = 1 - 0.39$  for  $gg$ . The selection coefficients are  $s_1 = 0.02$  for  $Gg$  and  $s_2 = 0.39$  for  $gg$ .

- 20.20** In a large randomly mating population, 0.84 of the individuals express the phenotype of the dominant allele  $A$  and 0.16 express the phenotype of the recessive allele  $a$ . (a) What is the frequency of the dominant allele? (b) If the  $aa$  homozygotes are 5 percent less fit than the other two genotypes, what will the frequency of  $A$  be in the next generation?

**ANS:** Frequency of  $a$  is  $q = 0.4$ ; (a) thus  $p = 1 - q = 0.6$ ; (b) use the following scheme:

Genotype	$AA$	$Aa$	$Aa$
Hardy-Weinberg frequency	0.36	0.48	0.16
Relative fitness	1	1	0.95
Relative contribution to next generation	0.36	0.48	0.152
Proportional contribution to next generation	0.363	0.484	0.153

Thus, the frequency of the  $A$  allele in the next generation will be  $(0.363 + 0.484/2) = 0.605$ .

- 20.21** Because individuals with cystic fibrosis die before they can reproduce, the coefficient of selection against them is  $s = 1$ . Assume that heterozygous carriers of the recessive mutant allele responsible for this disease are as fit as wild-type homozygotes and that the population frequency of the mutant allele is 0.02. (a) Predict the incidence of cystic fibrosis in the population after one generation of selection. (b) Explain why the incidence of cystic fibrosis hardly changes even with  $s = 1$ .

**ANS:** (a) Use the following scheme:

Genotype	<i>CC</i>	<i>Cc</i>	<i>cc</i>
Hardy–Weinberg frequency	$(0.98)^2 = 0.9604$	$2(0.98)(0.02) = 0.0392$	$(0.02)^2 = 0.0004$
Relative fitness	1	1	0
Relative contribution to next generation	$(0.9604) \times 1$	$(0.0392) \times 1$	0
Proportional contribution	$0.9604/0.9996 = 0.9608$	$0.0392/0.9996 = 0.0392$	0

The new frequency of the allele for cystic fibrosis is  $(0.5)(0.0392) = 0.0196$ ; thus, the incidence of the disease will be  $(0.0196)^2 = 0.00038$ , which is very slightly less than the incidence in the previous generation. (b) The incidence of cystic fibrosis does not change much because selection can only act against the recessive allele when it is in homozygotes, which are rare in the population.

**20.22** For each set of relative fitnesses for the genotypes *AA*, *Aa*, and *aa*, explain how selection is operating. Assume that  $0 < t < s < 1$ .

	<i>AA</i>	<i>Aa</i>	<i>aa</i>
Case 1	1	1	$1 - s$
Case 2	$1 - s$	$1 - s$	1
Case 3	1	$1 - t$	$1 - s$
Case 4	$1 - s$	1	$1 - t$

**ANS:** Case 1: selection is operating against a deleterious recessive allele. Case 2: selection is operating against a deleterious dominant allele. Case 3: selection is operating against a deleterious allele that has some expression in heterozygotes, that is, it is partially dominant. Case 4: selection is operating against both alleles in homozygous condition; this is a case of balancing selection.

**20.23** The frequency of newborn infants homozygous for a recessive lethal allele is about 1 in 25,000. What is the expected frequency of carriers of this allele in the population?

**ANS:**  $q^2 = 4 \times 10^{-5}$ ; thus  $q = 6.3 \times 10^{-3}$  and  $2pq = 0.0126$ .

**20.24** A population of size 50 reproduces in such a way that the population size remains constant. If mating is random, how rapidly will genetic variability, as measured by the frequency of heterozygotes, be lost from this population?

**ANS:** The frequency of heterozygotes will decrease by a  $1/(2N) = 1/100 = 0.01$  per generation.

**20.25** A population is segregating three alleles,  $A_1$ ,  $A_2$ , and  $A_3$ , with frequencies 0.2, 0.5, and 0.3, respectively. If these alleles are selectively neutral, what is the probability that  $A_2$  will ultimately be fixed by genetic drift? What is the probability that  $A_3$  will ultimately be lost by genetic drift?

**ANS:** Probability of ultimate fixation of  $A_2$  is 0.5; probability of ultimate loss of  $A_3$  is  $1 - 0.3 = 0.7$ .

**20.26** A small island population of mice consists of roughly equal numbers of males and females. The Y chromosome in one-fourth of the males is twice as long as the Y chromosome in the other males because of an expansion of heterochromatin. If mice with the large Y chromosome have the same fitness as mice with the small Y chromosome, what is the probability that the large Y chromosome will ultimately be fixed in the mouse population?

**ANS:** 0.25.

**20.27** In some regions of west Africa, the frequency of the  $HBB^S$  allele is 0.2. If this frequency is the result of a dynamic equilibrium due to the superior fitness of  $HBB^S HBB^A$  heterozygotes, and if  $HBB^S HBB^S$  homozygotes are essentially lethal, what is the intensity of selection against the  $HBB^A HBB^A$  homozygotes?

**ANS:**  $p = 0.2$ ; at equilibrium,  $p = t/(s + t)$ . Because  $s = 1$ , we can solve for  $t$ ;  $t = 0.25$ .

**20.28** Mice with the genotype *Hh* are twice as fit as either of the homozygotes *HH* and *hh*. With random mating, what is the expected frequency of the *b* allele when the mouse population reaches a dynamic equilibrium because of balancing selection?

**ANS:** The relative fitnesses of the genotypes *HH*, *Hh*, and *hh* are 0.5, 1, and 0.5, respectively. At equilibrium, the frequency of *b* will be  $s/(t + s) = (0.5)/(0.5 + 0.5) = 0.5$ .

**20.29** A completely recessive allele *g* is lethal in homozygous condition. If the dominant allele *G* mutates to *g* at a rate of  $10^{-6}$  per generation, what is the expected frequency of the lethal allele when the population reaches mutation–selection equilibrium?

**ANS:** At mutation–selection equilibrium

$$q = \sqrt{u/s} = \sqrt{10^{-6}/1} = 0.001.$$

**20.30** Individuals with the genotype *bb* are 20 percent less fit than individuals with the genotypes *BB* or *Bb*. If *B* mutates to *b* at a rate of  $10^{-6}$  per generation, what is the expected frequency of the allele *b* when the population reaches mutation–selection equilibrium?

**ANS:**  $q = \sqrt{u/s} = \sqrt{10^{-6}/(0.2)} = 2.2 \times 10^{-3}$ .

## CHAPTER 21

**21.1** Which of the following pairs of DNA sequences could qualify as the terminal repeats of a bacterial IS element. Explain.

- 5'-GAATCCGCA-3' and 5'-ACGCCTAAG-3'
- 5'-GAATCCGCA-3' and 5'-CTTAGGCGT-3'
- 5'-GAATCCGCA-3' and 5'-GAATCCGCA-3'
- 5'-GAATCCGCA-3' and 5'-TGCGGATTC-3'

**ANS:** The pair in (d) are inverted repeats and could therefore qualify.

**21.2** Which of the following pairs of DNA sequences could qualify as target site duplications at the point of an IS50 insertion? Explain.

(a) 5'-AATTCGCGT-3' and 5'-AATTCGCGT-3'

(b) 5'-AATTCGCGT-3' and 5'-TGCGCTTAA-3'

(c) 5'-AATTCGCGT-3' and 5'-TTAAGCGCA-3'

(d) 5'-AATTCGCGT-3' and 5'-ACGCGAATT-3'.

**ANS:** The pair in (a) are direct repeats and could therefore qualify.

**21.3** One strain of *E. coli* is resistant to the antibiotic streptomycin, and another strain is resistant to the antibiotic ampicillin. The two strains were cultured together and then plated on selective medium containing streptomycin and ampicillin. Several colonies appeared, indicating that cells had acquired resistance to both antibiotics. Suggest a mechanism to explain the acquisition of double resistance.

**ANS:** Resistance for the second antibiotic was acquired by conjugative gene transfer between the two types of cells.

**21.4** What distinguishes IS and Tn3 elements in bacteria?

**ANS:** Tn3 elements carry a gene that is not essential for transposition.

**21.5** The circular order of genes on the *E. coli* chromosome is \*A B C D E F G H\*, with the \* indicating that the ends of the chromosome are attached to each other. Two copies of an IS element are located in this chromosome: one between genes C and D, and the other between genes D and E. A single copy of this element is also present in the F plasmid. Two Hfr strains were obtained by selecting for integration of the F plasmid into the chromosome. During conjugation, one strain transfers the chromosomal genes in the order D E F G H A B C, whereas the other transfers them in the order D C B A H G F E. Explain the origin of these two Hfr strains. Why do they transfer genes in different orders? Does the order of transfer reveal anything about the orientation of the IS elements in the *E. coli* chromosome?

**ANS:** In the first strain, the F factor integrated into the chromosome by recombination with the IS element between genes C and D. In the second strain, it integrated by recombination with the IS element between genes D and E. The two strains transfer their genes in different orders because the two chromosomal IS elements are in opposite orientation.

**21.6** The composite transposon Tn5 consists of two IS50 elements, one on either side of a group of three genes for antibiotic resistance. The entire unit IS50L *kan<sup>r</sup> ble<sup>r</sup> str<sup>r</sup>* IS50R can transpose to a new location in the *E. coli* chromosome. However, of the two IS50 elements in this

transposon, only IS50R produces the catalytically active transposase. Would you expect IS50R to be able to be excised from the Tn5 composite transposon and insert elsewhere in the chromosome? Would you expect IS50L to be able to do this?

**ANS:** Both IS50 elements should be able to excise from the transposon and insert elsewhere in the chromosome, because even though IS50L does not produce its own transposase, IS50R provides a source of this enzyme.

**21.7** By chance, an IS1 element has inserted near an IS2 element in the *E. coli* chromosome. The gene between them, *sug<sup>+</sup>*, confers the ability to metabolize certain sugars. Will the unit IS1 *sug<sup>+</sup>* IS2 behave as a composite transposon? Explain.

**ANS:** No. IS1 and IS2 are mobilized by different transposases.

**21.8** A researcher has found a new Tn5 element with the structure IS50L *str<sup>r</sup> ble<sup>r</sup> kan<sup>r</sup>* IS50L. What is the most likely origin of this element?

**ANS:** IS50L inserted on each side of the cluster of antibiotic resistance genes.

**21.9** Would a Tn3 element with a frameshift mutation early in the *tnpA* gene be able to form a cointegrate? Would a Tn3 element with a frameshift mutation early in the *tnpR* gene be able to form a cointegrate?

**ANS:** The *tnpA* mutation: no; the *tnpR* mutation: yes.

**21.10** What enzymes are necessary for replicative transposition of Tn3? What are their respective functions?

**ANS:** Two enzymes, transposase and resolvase, are needed for replicative transposition. These enzymes are encoded by genes of Tn3. Transposase catalyzes formation of a cointegrate between donor and recipient plasmids. During this process, Tn3 is replicated so that there is a copy of it at each junction in the cointegrate. Resolvase catalyzes the site-specific recombination between the two Tn3 elements and thereby resolves the cointegrate, generating two molecules each with a copy of the transposon. Resolvase also represses the synthesis of both the transposase and resolvase enzymes.

**21.11** What is the medical significance of bacterial transposons?

**ANS:** Many bacterial transposons carry genes for antibiotic resistance, and it is relatively simple for these genes to move from one DNA molecule to another. DNA molecules that acquire resistance genes can be passed to other cells in a bacterial population, both vertically (by descent) and horizontally (by conjugative transfer). Over time, continued exposure to an antibiotic will select for cells that have acquired a gene for resistance to that antibiotic. The antibiotic will therefore no longer be useful in combating these bacteria.

**21.12** Describe the structure of the *Ac* transposon in maize. In what ways do the *Ds* transposons differ structurally and functionally from the *Ac* transposon?

**ANS:** The *Ac* element consists of 4563 nucleotide pairs bounded by inverted repeats that are 11 nucleotide pairs long. The *Ac* element is flanked by direct repeats of eight nucleotide pairs long; however, these repeats are created at the time the element is inserted into a chromosome (target site duplications) and are therefore not considered to be integral parts of the element itself. *Ds* elements possess the same terminal inverted repeats as *Ac*, but their internal sequences vary. Some residue of the *Ac* sequence may be present, or non-*Ac* sequences may be present; sometimes, one *Ds* element is contained within another *Ds* element.

**21.13** In homozygous condition, a deletion mutation of the *c* locus,  $c^{\Delta}$ , produces colorless (white) kernels in maize; the dominant wild-type allele, *C*, causes the kernels to be purple. A newly identified recessive mutation of the *c* locus,  $c^m$ , has the same phenotype as the deletion mutation (white kernels), but when  $c^m c^m$  and  $c^{\Delta} c^{\Delta}$  plants are crossed, they produce white kernels with purple stripes. If it is known that the  $c^{\Delta} c^{\Delta}$  plants harbor *Ac* elements, what is the most likely explanation for the  $c^m$  mutation?

**ANS:** The  $c^m$  mutation is due to a *Ds* or an *Ac* insertion.

**21.14** In maize, the *O2* gene, located on chromosome 7, controls the texture of the endosperm, and the *C* gene, located on chromosome 9, controls its color. The gene on chromosome 7 has two alleles, a recessive, *o2*, which causes the endosperm to be soft, and a dominant, *O2*, which causes it to be hard. The gene on chromosome 9 also has two alleles, a recessive, *c*, which allows the endosperm to be colored, and a dominant, *C*<sup>l</sup>, which inhibits coloration. In one homozygous *C*<sup>l</sup> strain, a *Ds* element is inserted on chromosome 9 between the *C* gene and the centromere. This element can be activated by introducing an *Ac* element by appropriate crosses. Activation of *Ds* causes the *C*<sup>l</sup> allele to be lost by chromosome breakage. In *C*<sup>l</sup>/*c*/*c* kernels, such loss produces patches of colored tissue in an otherwise colorless background. A geneticist crosses a strain with the genotype *o2/o2*; *C*<sup>l</sup> *Ds*/*C*<sup>l</sup> *Ds* to a strain with the genotype *O2/o2*; *c/c*. The latter strain also carries an *Ac* element somewhere in the genome. Among the offspring, only those with hard endosperm show patches of colored tissue. What does this tell you about the location of the *Ac* element in the *O2/o2*; *c/c* strain?

**ANS:** The *Ac* element must be tightly linked to the *O2* allele.

**21.15** In maize, the recessive allele *bz* (*bronze*) produces a lighter color in the aleurone than does the dominant allele, *Bz*. Ears on a homozygous *bz/bz* plant were fertilized by pollen from a homozygous *Bz/Bz* plant. The resulting cobs contained kernels that were uniformly dark except for a few on which light spots occurred. Suggest an explanation.

**ANS:** The paternally inherited *Bz* allele was inactivated by a transposable element insertion.

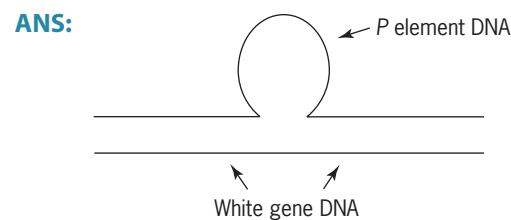
**21.16** The X-linked *singed* locus is one of several in *Drosophila* that controls the formation of bristles on the adult cuticle. Males that are hemizygous for a mutant *singed* allele have bent, twisted bristles that are often much reduced in size. Several *P* element insertion mutations of the *singed* locus have been characterized, and some have been shown to revert to the wild-type allele by excision of the inserted element. What conditions must be present to allow such reversions to occur?

**ANS:** The *P* transposase to catalyze excision and the absence of *P*-specific piRNAs that would repress excision.

**21.17** Dysgenic hybrids in *Drosophila* have elevated mutation rates as a result of *P* element transposition. How could you take advantage of this situation to obtain *P* element insertion mutations on the X chromosome?

**ANS:** Cross dysgenic (highly mutable) males carrying a wild-type X chromosome to females homozygous for a balancer X chromosome; then cross the heterozygous F<sub>1</sub> daughters individually to their brothers and screen the F<sub>2</sub> males that lack the balancer chromosome for mutant phenotypes, including failure to survive (lethality). Mutations identified in this screen are probably due to *P* element insertions in X-linked genes.

**21.18** If DNA from a *P* element insertion mutation of the *Drosophila white* gene and DNA from a wild-type *white* gene were purified, denatured, mixed with each other, reannealed, and then viewed with an electron microscope, what would the hybrid DNA molecules look like?



(Should see a single-stranded DNA loop corresponding to the insertion.)

**21.19** When complete *P* elements are injected into embryos from an M strain, they transpose into the chromosomes of the germ line, and progeny reared from these embryos can be used to establish new *P* strains. However, when complete *P* elements are injected into embryos from insects that lack these elements, such as mosquitoes, they do not transpose into the chromosomes of the germ line. What does this failure to insert in the chromosomes of other insects indicate about the nature of *P* element transposition?

**ANS:** Factors made by the fly's genome are required for transposition; other insects apparently lack the ability to provide these factors.

**21.20** (a) What are retroviruslike elements? (b) Give examples of retroviruslike elements in yeast and *Drosophila*.

(c) Describe how retroviruslike elements transpose.  
 (d) After a retroviruslike element has been inserted into a chromosome, is it ever expected to be excised?

**ANS:** (a) Retroviruslike elements resemble integrated retroviruses in overall structure and behavior. (b) Examples include the *Ty1* element in yeast and the *copia* element in *Drosophila*. (c) Retroviruslike elements transpose using an RNA intermediate. The element DNA is transcribed into single-stranded RNA, which is reverse-transcribed into double-stranded DNA (cDNA). The double-stranded cDNA is then inserted into a site in the genome. (d) No. However, the LTRs could pair and recombine to excise all but one LTR.

**21.21** Sometimes, solitary copies of the LTR of *Ty1* elements are found in yeast chromosomes. How might these solitary LTRs originate?

**ANS:** Through crossing over between the LTRs of a *Ty1* element.

**21.22** Would you ever expect the genes in a retrotransposon to possess introns? Explain.

**ANS:** No. The intron sequences would be removed by RNA processing prior to reverse transcription into DNA.

**21.23** Suggest a method to determine whether the *TART* retroposon is situated at the telomeres of each of the chromosomes in the *Drosophila* genome.

**ANS:** *In situ* hybridization to polytene chromosomes using a *TART* probe.

**21.24** It has been proposed that the *bobo* transposable elements in *Drosophila* mediate intrachromosomal recombination—that is, two *bobo* elements on the same chromosome pair and recombine with each other. What would such a recombination event produce if the *bobo* elements were oriented in the same direction on the chromosome? What if they were oriented in opposite directions?

**ANS:** Same orientation: a deletion; opposite orientation: an inversion.

**21.25** What evidence suggests that some transposable elements are not simply genetic parasites?

**ANS:** *TART* and *HeT-A* replenish the ends of *Drosophila* chromosomes.

**21.26** Approximately half of all spontaneous mutations in *Drosophila* are caused by transposable element insertions. In human beings, however, the accumulated evidence suggests that the vast majority of spontaneous mutations are *not* caused by transposon insertions. Propose a hypothesis to explain this difference.

**ANS:** The transposition rate in humans may be very much less than it is in *Drosophila*.

**21.27** Z. Ivics, Z. Izsvák, and P. B. Hackett have “resurrected” a nonmobile member of the *Tc1/mariner* family of transposable elements isolated from the DNA of salmon.

These researchers altered 12 codons within the coding sequence of the transposase gene of the salmon element to restore the catalytic function of its transposase. The altered element, called *Sleeping Beauty*, is being tested as an agent for the genetic transformation of vertebrates such as mice and zebra fish (and possibly humans). Suppose that you have a bacterial plasmid containing the gene for green fluorescent protein (*gfp*) inserted between the ends of a *Sleeping Beauty* element. How would you go about obtaining mice or zebra fish that express the *gfp* gene?

**ANS:** The *Sleeping Beauty* element could be used as a transformation vector in vertebrates much like the *P* element has been used in *Drosophila*. The *gfp* gene could be inserted between the ends of the *Sleeping Beauty* element and injected into eggs or embryos along with an intact *Sleeping Beauty* element capable of encoding the element’s transposase. If the transposase that is produced in the injected egg or embryo acts on the element that contains the *gfp* gene, it might cause the latter to be inserted into genomic DNA. Then, if the egg or embryo develops into an adult, that adult can be bred to determine if a *Sleeping Beauty/gfp* transgene is transmitted to the next generation. In this way, it would be possible to obtain strains of mice or zebra fish that express the *gfp* gene.

**21.28** The human genome contains about 5000 “processed pseudogenes,” which are derived from the insertion of DNA copies of mRNA molecules derived from many different genes. Predict the structure of these pseudogenes. Would each type of processed pseudogene be expected to found a new family of retrotransposons within the human genome? Would the copy number of each type of processed pseudogene be expected to increase significantly over evolutionary time, as the copy number of the *Alu* family has? Explain your answers.

**ANS:** The processed pseudogenes will, in the best of cases, contain sequences from the transcription start site to the poly-A tail of the transcript (if there is one); however, they will not contain the gene’s promoter or any of its introns. Because these pseudogenes will not have a promoter, they are not likely to found new retrotransposon families. Without a promoter, they will not be transcribed; hence, they will not produce RNA to be reverse transcribed into DNA for insertion into other sites in the genome. Most likely, the copy number of each of these processed pseudogenes will not increase as the copy number of the *Alu* element has. The *Alu* element contains an “internal” promoter recognized by RNA polymerase III. Each insertion of the *Alu* element contains this promoter and can therefore be transcribed into RNA, which can subsequently be reverse transcribed into DNA. The *L1* element also contains an “internal” promoter, but this promoter is recognized by RNA polymerase II. Most protein-coding genes contain an “external” promoter—that is, one that is not transcribed—and this promoter is recognized by RNA polymerase II.

## CHAPTER 22

- 22.1** During oogenesis, what mechanisms enrich the cytoplasm of animal eggs with nutritive and determinative materials?
- ANS:** Unequal division of the cytoplasm during the meiotic divisions; transport of substances into the oocyte from surrounding cells such as the nurse cells in *Drosophila*.
- 22.2** Predict the phenotype of a fruit fly that develops from an embryo in which the posterior pole cells had been destroyed by a laser beam.
- ANS:** The fly will be sterile because the posterior pole cells form the germ line in adults of both sexes.
- 22.3** Outline the main steps in the genetic analysis of development in a model organism such as *Drosophila*.
- ANS:** Collect mutations with diagnostic phenotypes; map the mutations and test them for allelism with one another; perform epistasis tests with mutations in different genes; clone individual genes and analyze their function at the molecular level.
- 22.4** Why is the early *Drosophila* embryo a syncytium?
- ANS:** Mitotic division is so rapid that there is not enough time for membranes to form between cells.
- 22.5** In *Drosophila*, what larval tissues produce the external organs of the adult?
- ANS:** Imaginal discs.
- 22.6** Like *dorsal*, *bicoid* is a strict maternal-effect gene in *Drosophila*; that is, it has no zygotic expression. Recessive mutations in *bicoid* (*bcd*) cause embryonic death by preventing the formation of anterior structures. Predict the phenotypes of (a) *bcd/bcd* animals produced by mating heterozygous males and females; (b) *bcd/bcd* animals produced by mating *bcd/bcd* females with *bcd/+* males; (c) *bcd/+* animals produced by mating *bcd/bcd* females with *bcd/+* males; (d) *bcd/bcd* animals produced by mating *bcd/+* females with *bcd/bcd* males; (e) *bcd/+* animals produced by mating *bcd/+* females with *bcd/bcd* males.
- ANS:** (a) Wild-type; (b) embryonic lethal; (c) embryonic lethal; (d) wild-type; (e) wild-type.
- 22.7** Why do women, but not men, who are homozygous for the mutant allele that causes phenylketonuria produce children that are physically and mentally retarded?
- ANS:** In homozygous condition, the mutation that causes phenylketonuria has a maternal effect. Women homozygous for this mutation influence the development of their children *in utero*.
- 22.8** In *Drosophila*, recessive mutations in the dorsal–ventral axis gene *dorsal* (*dl*) cause a dorsalized phenotype in embryos produced by *dl/dl* mothers; that is, no ventral structures develop. Predict the phenotype of embryos produced by females homozygous for a recessive mutation in the anterior–posterior axis gene *nanos*.
- ANS:** Some structures fail to develop in the posterior portion of the embryo.
- 22.9** A researcher is planning to collect mutations in maternal-effect genes that control the earliest events in *Drosophila* development. What phenotype should the researcher look for in this search for maternal-effect mutations?
- ANS:** Female sterility. Females affected by these mutations will lay abnormal eggs that will not develop into viable embryos.
- 22.10** A researcher is planning to collect mutations in the gap genes, which control the first steps in the segmentation of *Drosophila* embryos. What phenotype should the researcher look for in this search for gap gene mutations?
- ANS:** Screen for lethal mutations that prevent regions of the embryo from developing normally.
- 22.11** How do the somatic cells that surround a developing *Drosophila* egg in the ovary influence the formation of the dorsal–ventral axis in the embryo that will be produced after the egg is fertilized?
- ANS:** The somatic cells surrounding a developing *Drosophila* egg in the ovary determine where the spätzle protein, which is the ligand for the Toll receptor protein, will be cleaved. This cleavage will eventually occur on the ventral side of the developing embryo.
- 22.12** What events lead to a high concentration of hunchback protein in the anterior of *Drosophila* embryos?
- ANS:** The *hunchback* mRNA is translated into protein only in the anterior region of the developing embryo. This RNA is supplied to the egg by the nurse cells and it is also synthesized after fertilization by transcription of the *hunchback* gene. This zygotic transcription is stimulated by a transcription factor encoded by maternally supplied *bicoid* mRNA, which is located in the anterior of the egg. Thus, *hunchback* mRNA is concentrated in the anterior of the embryo. In addition, the *hunchback* mRNA that is located in the posterior of the embryo is bound by nanos protein and then degraded. The nanos protein is concentrated in the posterior of the embryo because maternally supplied *nanos* mRNA is preferentially localized there.
- 22.13** Diagram a pathway that shows the contributions of the *sevenless* (*sev*) and *bride of sevenless* (*boss*) genes to the differentiation of the R7 photoreceptor in the ommatidia of *Drosophila* eyes. Where would *eyeless* (*ey*) fit in this pathway?
- ANS:**  $ey \rightarrow boss \rightarrow sev \rightarrow R7$  differentiation
- 22.14** The *sev*<sup>B4</sup> allele is temperature sensitive; at 22.7°C, flies that are homozygous for it develop normal R7 photoreceptors, but at 24.3°C, they fail to develop these photoreceptors. *sos*<sup>2A</sup> is a recessive, loss-of-function mutation in the *son of sevenless* (*sos*) gene. Flies with the genotype



*sev<sup>B4</sup>/sev<sup>B4</sup>; sos<sup>2A</sup>/+* fail to develop R7 photoreceptors if they are raised at 22.7°C. Therefore, *sos2A* acts as a dominant enhancer of the *sev<sup>B4</sup>* mutant phenotype at this temperature. Based on this observation, where is the protein product of the wild-type *sos* gene—called SOS—likely to act in the pathway for R7 differentiation?

**ANS:** If the SEV protein is activated—either by the BOSS ligand or by a gain-of-function mutation in the *sev* gene, a faulty effector protein could stop it from inducing the R7 cell to differentiate. The SOS protein is likely to be a downstream effector in the pathway for R7 differentiation because when it is depleted by mutating one copy of the *sos* gene, flies that have a partially functional SEV protein show a mutant phenotype—that is, transmission of the developmental signal through SEV and its downstream effector proteins is weakened.

**20.15** When the mouse *Pax6* gene, which is homologous to the *Drosophila eyeless* gene, is expressed in *Drosophila*, it produces extra compound eyes with ommatidia, just like normal *Drosophila* eyes. If the *Drosophila eyeless* gene were introduced into mice and expressed there, what effect would you expect? Explain.

**ANS:** Because the *Pax6* gave the same phenotype in flies as overexpression of the *eyeless* gene, the genes must be functionally homologous, as well as structurally homologous. Therefore, expect extra mouse eyes or eye primordia when expressing *eyeless* in the mouse.

**22.16** Would you expect to find homologues of *Drosophila*'s BX-C and ANT-C genes in animals with radial symmetry such as sea urchins and starfish? How could you address this question experimentally?

**ANS:** Maybe these organisms would not have homologues of the BX-C and ANT-C genes because they do not have segmented bodies with bilateral symmetry as *Drosophila* does. To see if homologues to these genes are present, use *Drosophila* BX-C and ANT-C DNA as probes to hybridize with starfish or sea urchin genomic DNA on a Southern blot. The hybridization would have to be done under conditions that allow DNA that is not a perfect match to form a duplex—that is, under conditions of low stringency. Usually, hybridizations of this type are carried out at lower temperatures than typical Southern hybridizations. If the probes stick to the DNA on the blot, there is evidence for homologues to the BX-C and ANT-C genes in the genomic DNA. Follow-up experiments might endeavor to clone this DNA and, ultimately, to sequence it to determine just how close a match it is to the *Drosophila* probe DNA.

**22.17** How might you show that two mouse *Hox* genes are expressed in different tissues and at different times during development?

**ANS:** Northern blotting of RNA extracted from the tissues at different times during development. Hybridize the blot with gene-specific probes.

**22.18** Distinguish between therapeutic and reproductive cloning.

**ANS:** Therapeutic cloning involves the creation of an embryo by implanting the nucleus of a somatic cell into an enucleated egg and stimulating the egg to divide. Stem cells are then taken from the embryo to differentiate into specific tissues in the individual from which the somatic cell was taken. These tissues will be genetically identical to the other tissues of the individual—thus, they are unlikely to be rejected by the individual's immune system. Reproductive cloning involves the creation of an embryo by implanting the nucleus of a somatic cell into an enucleated egg and then allowing the egg to develop into an entire individual.

**22.19** What is the scientific significance of reproductive cloning?

**ANS:** Reproductive cloning of mammals such as sheep, mice, and cats indicates that somatic cell nuclei have all the genetic information to direct the development of a complete, viable organism. It also shows that epigenetic modifications of chromatin, such as X chromosome inactivation, can be reset.

**22.20** The methylation of DNA, the acetylation of histones, and the packaging of DNA into chromatin by certain kinds of proteins are sometimes referred to as epigenetic modifications of the DNA. These modifications portend difficulties for reproductive cloning. Do they also portend difficulties for therapeutic cloning and for the use of stem cells to treat diseases or injuries that involve the loss of specific cell types?

**ANS:** Methylation of DNA, acetylation of histones, and packaging of DNA into chromatin by certain kinds of proteins all portend difficulties for therapeutic cloning as well as for reproductive cloning. These epigenetic modifications of somatic cell DNA would have to be “reprogrammed” in the oocyte or they could affect how the stem cells derived from the oocyte would develop.

**22.21** Assume that an animal is capable of producing 100 million different antibodies and that each antibody contains a light chain of 220 amino acids long and a heavy chain of 450 amino acids. How much genomic DNA would be needed to accommodate the coding sequences of these genes?

**ANS:** If each antibody consists of one kind of light chain and one kind of heavy chain, and if light and heavy chains can combine freely, the potential to produce 100 million different antibodies implies the existence of 10,000 light chain genes and 10,000 heavy chain genes ( $10,000 \times 10,000 = 100$  million). If each light chain is 220 amino acids long, each light chain gene must comprise  $3 \times 220 = 660$  nucleotides because each amino acid is specified by a triplet of nucleotides; similarly, each heavy chain gene must comprise  $3 \times 450 = 1350$  nucleotides. Therefore, the genome must contain  $10,000 \times 660 = 6.6$  million nucleotides devoted to light chain production and

$10,000 \times 1,350 = 13.5$  million nucleotides devoted to heavy chain production. Altogether, then, the genome must contain 19.5 million nucleotides dedicated to encoding the amino acids of the various antibody chains.

**22.22** Each  $L_kV_k$  gene segment in the kappa light chain locus on chromosome 2 consists of two coding exons, one for the leader peptide and one for the variable portion of the kappa light chain. Would you expect to find a stop codon at the end of the coding sequence in the second ( $V_k$ ) exon?

**ANS:** No, because the  $V_k$  coding sequence must be joined to the coding sequence of the constant region to encode a complete kappa light chain.

## CHAPTER 23

**23.1** Many cancers seem to involve environmental factors. Why, then, is cancer called a genetic disease?

**ANS:** Cancer has been called a genetic disease because it results from mutations of genes that regulate cell growth and division. Nonhereditary forms of cancer result from mutations in somatic cells. These mutations, however, can be induced by environmental factors including tobacco smoke, chemical pollutants, ionizing radiation, and UV light. Hereditary forms of cancer also frequently involve the occurrence of environmentally induced somatic mutations.

**23.2** Both embryonic cells and cancer cells divide quickly. How can these two types of cells be distinguished from each other?

**ANS:** Cancer cells do not display contact inhibition—they pile up on top of each other—whereas embryonic cells spread out in flat sheets. Cancer cells are frequently aneuploid; embryonic cells are euploid.

**23.3** Most cancer cells are aneuploid. Suggest how aneuploidy might contribute to deregulation of the cell cycle.

**ANS:** Aneuploidy might involve the loss of functional copies of tumor suppressor genes, or it might involve the inappropriate duplication of proto-oncogenes. Loss of tumor suppressor genes would remove natural brakes on cell division, and duplication of proto-oncogenes would increase the abundance of factors that promote cell division.

**23.4** Would you ever expect to find a tumor-inducing retrovirus that carried a processed cellular tumor suppressor gene in its genome?

**ANS:** No. A virus that carried a processed copy of a tumor suppressor gene would not be expected to induce tumor formation because the product of the tumor suppressor gene would help to restrain cell growth and division.

**23.5** How do we know that normal cellular oncogenes are not simply integrated retroviral oncogenes that have acquired the proper regulation?

**ANS:** They possess introns.

**23.6** How might the absence of introns in a retroviral oncogene explain that gene's overexpression in the tissues of an infected animal?

**ANS:** The absence of introns might speed up the expression of the gene's protein product because there would be no need for splicing. In addition, some introns contain sequences called silencers that negatively regulate transcription. Removal of these sequences might cause transcription to occur when it otherwise would not.

**23.7** When cellular oncogenes are isolated from different animals and compared, the amino acid sequences of the polypeptides they encode are found to be very similar. What does this suggest about the functions of these polypeptides?

**ANS:** The products of these genes play important roles in cell activities.

**23.8** The majority of the *c-ras* oncogenes obtained from cancerous tissues have mutations in codon 12, 59, or 61 in the coding sequence. Suggest an explanation.

**ANS:** Mutations in these codons cause amino acid changes that activate the Ras protein.

**23.9** When a mutant *c-H-ras* oncogene with a valine for glycine substitution in codon 12 is transfected into cultured NIH 3T3 cells, it transforms those cells into cancer cells. When the same mutant oncogene is transfected into cultured embryonic cells, it does not transform them. Why?

**ANS:** The cultured NIH 3T3 cells probably carry other mutations that predispose them to become cancerous; transfection of such cells with a mutant *c-H-ras* oncogene may be the last step in the process of transforming the cells into cancer cells. Cultured embryonic cells probably do not carry the predisposing mutations needed for them to become cancerous; thus, when they are transfected with the mutant *c-H-ras* oncogene, they continue to divide normally.

**23.10** A mutation in the *ras* cellular oncogene can cause cancer when it is in heterozygous condition, but a mutation in the *RB* tumor suppressor gene can cause cancer only when it is in homozygous condition. What does this difference between dominant and recessive mutations imply about the roles that the *ras* and *RB* gene products play in normal cellular activities?

**ANS:** Ras protein is an activator of cell division, whereas RB protein is a suppressor of cell division.

**23.11** Explain why individuals who develop nonhereditary retinoblastoma usually have tumors in only one eye, whereas individuals with hereditary retinoblastoma usually develop tumors in both eyes.

**ANS:** Retinoblastoma results from homozygosity for a loss-of-function (recessive) allele. The sporadic occurrence of retinoblastoma requires two mutations of this gene in the same cell or cell lineage. Therefore, retinoblastoma is rare among individuals who, at conception, are

homozygous for the wild-type allele of the *RB* gene. For such individuals, we would expect the frequency of tumors in both eyes to be the square of the frequency of tumors in one eye. Individuals who are heterozygous for a mutant *RB* allele require only one somatic mutation to occur for them to develop retinoblastoma. Because there are millions of cells in each retina, there is a high probability that this somatic mutation will occur in at least one cell in each eye, causing both eyes to develop tumors.

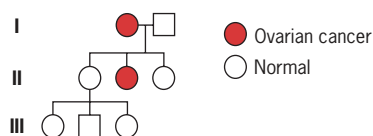
- 23.12** Approximately 5 percent of the individuals who inherit an inactivated *RB* gene do not develop retinoblastoma. Use this statistic to estimate the number of cell divisions that form the retinal tissues of the eye. Assume that the rate at which somatic mutations inactivate the *RB* gene is one mutation per  $10^6$  cell divisions.

**ANS:** The probability that a carrier does not develop retinoblastoma is 0.05, which is equal to the probability that the wild-type *RB* allele is not mutationally inactivated during the cell divisions that form the retinas of the eyes. If the rate of mutational inactivation is  $u = 10^{-6}$  per cell division, and  $n$  is the number of cell divisions, then  $0.05 = (1 - u)^n$ . If we take logarithms of both sides,  $\log(0.05) = n \log(1 - u)$ , which to a good approximation, is equal to  $n \log(u)$ . After substituting  $10^{-6}$  for  $u$  and solving, we find that  $n = 1.3 \times 10^6$ .

- 23.13** Inherited cancers like retinoblastoma show a dominant pattern of inheritance. However, the underlying genetic defect is a recessive loss-of-function mutation—often the result of a deletion. How can the dominant pattern of inheritance be reconciled with the recessive nature of the mutation?

**ANS:** At the cellular level, loss-of-function mutations in the *RB* gene are recessive; a cell that is heterozygous for such a mutation divides normally. However, when a second mutation occurs, that cell becomes cancerous. If the first *RB* mutation was inherited, there is a high probability that the individual carrying this mutation will develop retinoblastoma because a second mutation can occur any time during the formation of the retinas in either eye. Thus, the individual is predisposed to develop retinoblastoma, and it is this predisposition that shows a dominant pattern of inheritance.

- 23.14** The following pedigree shows the inheritance of familial ovarian cancer caused by a mutation in the *BRCA1* gene. Should II-1 be tested for the presence of the predisposing mutation? Discuss the advantages and disadvantages of testing.



**ANS:** II-1 should be tested for the *BRCA1* mutation that apparently was involved in the ovarian cancer that developed in her mother and sister. If she is found to carry this

mutation, a prophylactic oophorectomy can be prescribed to reduce the chance that she will develop cancer. If she is found to be free of the mutation carried by her mother and sister, then she is not more likely to develop ovarian cancer than a woman in the general population.

- 23.15** In what sense is pRB a negative regulator of E2F transcription factors?

**ANS:** By binding to E2F transcription factors, pRB prevents those transcription factors from activating their target genes—which encode proteins involved in progression of the cell cycle; pRB is therefore a negative regulator of transcription factors that stimulate cell division.

- 23.16** A particular E2F transcription factor recognizes the sequence TTTCGCGC in the promoter of its target gene. A temperature-sensitive mutation in the gene encoding this E2F transcription factor alters the ability of its protein product to activate transcription; at 25°C, the mutant protein activates transcription normally, but at 35°C, it fails to activate transcription at all. However, the ability of the protein to recognize its target DNA sequence is not impaired at either temperature. Would cells heterozygous for this temperature-sensitive mutation be expected to divide normally at 25°C? at 35°C? Would your answers change if the E2F protein functions as a homodimer?

**ANS:** At 25°, cell division should be normal—the same as for cells homozygous for a wild-type allele of the *E2F* gene. At 35°, division would be expected to be impaired either because the mutant E2F protein binds unproductively to the sequence in its target gene or because a mutant E2F polypeptide dimerizes with a wild-type E2F polypeptide and abolishes the activation function of the wild-type polypeptide.

- 23.17** During the cell cycle, the p16 protein is an inhibitor of cyclin/CDK activity. Predict the phenotype of cells homozygous for a loss-of-function mutation in the gene that encodes p16. Would this gene be classified as a proto-oncogene or as a tumor suppressor gene?

**ANS:** Cells homozygous for a loss-of-function mutation in the *p16* gene might be expected to divide in an uncontrolled manner because the p16 protein would not be able to inhibit cyclin-CDK activity during the cell cycle. The *p16* gene would therefore be classified as a tumor suppressor gene.

- 23.18** The *BCL-2* gene encodes a protein that represses the pathway for programmed cell death. Predict the phenotype of cells heterozygous for a dominant activating mutation in this gene. Would the *BCL-2* gene be classified as a proto-oncogene or as a tumor suppressor gene?

**ANS:** Cells heterozygous for a dominant activating mutation in the *BCL-2* gene would be expected to be unable to execute the programmed cell death pathway in response to DNA damage induced by radiation treatment. Such cells would continue to divide and accumulate

mutations; ultimately they would have a good chance of becoming cancerous. The *BCL-2* gene would therefore be classified as a proto-oncogene.

- 23.19** The protein product of the *BAX* gene negatively regulates the protein product of the *BCL-2* gene—that is, BAX protein interferes with the function of the BCL-2 protein. Predict the phenotype of cells homozygous for a loss-of-function mutation in the *BAX* gene. Would this gene be classified as a proto-oncogene or as a tumor suppressor gene?

**ANS:** Cells homozygous for a loss-of-function mutation in the *BAX* gene would be unable to prevent repression of the programmed cell death pathway by the BCL-2 gene product. Consequently, these cells would be unable to execute that pathway in response to DNA damage induced by radiation treatment. Such cells would continue to divide and accumulate mutations; ultimately, they would have a good chance of becoming cancerous. The *BAX* gene would therefore be classified as a tumor suppressor gene.

- 23.20** Cancer cells frequently are homozygous for loss-of-function mutations in the *TP53* gene, and many of these mutations map in the portion of *TP53* that encodes the DNA-binding domain of p53. Explain how these mutations contribute to the cancerous phenotype of the cells.

**ANS:** Loss-of-function mutations in the DNA-binding domain of p53 abolish the ability of that protein to activate transcription of target genes whose products are involved in the restraint of cell division or in the promotion of programmed cell death. Without restraint of cell division or promotion of programmed cell death, cells accumulate damage to their DNA and ultimately become cancerous.

- 23.21** Suppose that a cell is heterozygous for a mutation that caused p53 to bind tightly and constitutively to the DNA of its target genes. How would this mutation affect the cell cycle? Would such a cell be expected to be more or less sensitive to the effects of ionizing radiation?

**ANS:** If a cell were heterozygous for a mutation that caused p53 to bind tightly and constitutively to the DNA of its target genes, its growth and division might be retarded, or it might be induced to undergo apoptosis. Such a cell would be expected to be more sensitive to the effects of ionizing radiation because radiation increases the expression of p53, and in this case, the p53 would be predisposed to activate its target genes, causing the cell to respond vigorously to the radiation treatment.

- 23.22** Mice homozygous for a knockout mutation of the *TP53* gene are viable. Would they be expected to be more or less sensitive to the killing effects of ionizing radiation?

**ANS:** Homozygous *TP53* knockout mice might actually be less sensitive to the killing effects of ionizing radiation because p53 would be unable to mediate the apoptotic response to the radiation treatment.

- 23.23** Would cancer-causing mutations of the *APC* gene be expected to increase or decrease the ability of pAPC to bind  $\beta$ -catenin?

**ANS:** They would probably decrease the ability of pAPC to bind  $\beta$ -catenin.

- 23.24** Mice that are heterozygous for a knockout mutation in the *RB* gene develop pituitary and thyroid tumors. Mice that are homozygous for this mutation die during embryonic development. Mice that are homozygous for a knockout mutation in the gene encoding the p130 homologue of RB and heterozygous for a knockout mutation in the gene encoding the p107 homologue of RB do not have a tendency to develop tumors. However, homozygotes for knockout mutations in both of these genes die during embryonic development. What do these findings suggest about the roles of the *RB*, *p139*, and *p107* genes in embryos and adults?

**ANS:** All three genes (*RB*, *p130*, and *p107*) are essential for embryonic development, although by themselves, *p130* and *p107* are dispensable, possibly because their products are functionally redundant. (Both gene products must be inactivated before any deleterious effect is seen.) In adults, only pRB appears to play a role in suppressing tumor formation.

- 23.25** It has been demonstrated that individuals with diets poor in fiber and rich in fatty foods have an increased risk to develop colorectal cancer. Fiber-poor, fat-rich diets may irritate the epithelial lining of the large intestine. How could such irritation contribute to the increased risk for colorectal cancer?

**ANS:** The increased irritation to the intestinal epithelium caused by a fiber-poor, fat-rich diet would be expected to increase the need for cell division in this tissue (to replace the cells that were lost because of the irritation), with a corresponding increase in the opportunity for the occurrence of cancer-causing mutations.

- 23.26** Messenger RNA from the *KAI1* gene is strongly expressed in normal prostate tissues but weakly expressed in cell lines derived from metastatic prostate cancers. What does this finding suggest about the role of the *KAI1* gene product in the etiology of prostate cancer?

**ANS:** The *KAI1* gene is a prostate tumor suppressor gene. Functional inactivation of this gene allows prostate tumors to develop.

- 23.27** The p21 protein is strongly expressed in cells that have been irradiated. Researchers have thought that this strong expression is elicited by transcriptional activation of the *p21* gene by the p53 protein acting as a transcription factor. Does this hypothesis fit with the observation that p21 expression is induced by radiation treatment in mice homozygous for a knockout mutation in the *TP53* gene? Explain.

**ANS:** No. Apparently there is another pathway—one not mediated by p53—that leads to the activation of the *p21* gene.

CHAPTER 24

**24.1** What was some of the evidence that led Charles Darwin to argue that species change over time?

**ANS:** Among other things, Darwin observed species on islands that were different from each other and from continental species but were still similar enough to indicate that they were related. He also observed variation within species, especially within domesticated breeds, and saw how the characteristics of an organism could be changed by selective breeding. His observations of fossilized organisms indicated that some species have become extinct.

**24.2** Darwin stressed that species evolve by natural selection. What was the main gap in his theory?

**ANS:** Darwin did not understand the mechanism of inheritance; he did not know of Mendel's principles.

**24.3** Using the data in Table 24.1, and assuming that mating is random with respect to the blood type, predict the frequencies of the three genotypes of the Duffy blood-type locus in a South African and an English population.

**ANS:** The frequency of the *a* allele is 0.06 in the South African population and 0.42 in the English population. The predicted genotype frequencies under the assumption of random mating are as follows:

Genotype	South Africa	England
<i>aa</i>	$(0.06)^2 = 0.004$	$(0.42)^2 = 0.18$
<i>ab</i>	$2(0.06)(0.94) = 0.11$	$2(0.42)(0.58) = 0.49$
<i>bb</i>	$(0.94)^2 = 0.88$	$(0.58)^2 = 0.33$

**24.4** Theodosius Dobzhansky and his collaborators studied chromosomal polymorphisms in *Drosophila pseudoobscura* and its sister species in the western United States. In one study of polymorphisms in chromosome III of *D. pseudoobscura* sampled from populations at different locations in the Yosemite region of the Sierra Nevada, Dobzhansky (1948, *Genetics* 33: 158–176) recorded the following frequencies of the Standard (ST) banding pattern:

Location	Frequency ST	Elevation (in feet)
Jacksonville	0.46	850
Lost Claim	0.41	3,000
Mather	0.32	4,600
Aspen	0.26	6,200
Porcupine	0.14	8,000
Tuolumne	0.11	8,600
Timberline	0.10	9,900
Lyell Base	0.10	10,500

What is interesting about these data?

**ANS:** The frequency of the ST banding pattern declines with increasing altitude. Thus, the data indicate that this chromosomal polymorphism exhibits an altitudinal cline.

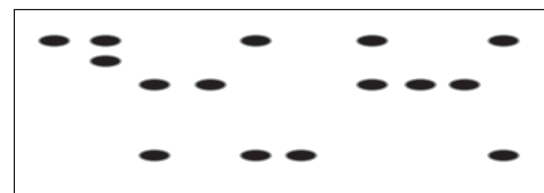
**24.5** In a survey of electrophoretically detectable genetic variation in the alcohol dehydrogenase gene of *Drosophila melanogaster*, a researcher found two allozymes, denoted F (fast) and S (slow) in a population; 32 individuals were homozygous for the *F* allele of the gene, 22 were homozygous for the *S* allele, and 46 were heterozygous for the *F* and *S* alleles. Are the observed frequencies of the three genotypes consistent with the assumption that the population is in Hardy–Weinberg equilibrium?

**ANS:** In the sample, the frequency of the *F* allele is  $(2 \times 32 + 46)/(2 \times 100) = 0.55$  and the frequency of the *S* allele is  $1 - 0.55 = 0.45$ . The predicted and observed genotype frequencies are as follows:

Genotype	Observed	Hardy–Weinberg Predicted
<i>FF</i>	32	$100 \times (0.55)^2 = 30.25$
<i>FS</i>	46	$100 \times 2(0.55)(0.45) = 49.5$
<i>SS</i>	22	$100 \times (0.45)^2 = 20.25$

To test for agreement between the observed and predicted values, we compute a chi-square statistic with 1 degree of freedom:  $\chi^2 = \Sigma(\text{obs.} - \text{pred.})^2/\text{pred.} = 0.50$ , which is not significant at the 5 percent level. Thus, the population appears to be in Hardy–Weinberg equilibrium for the alcohol dehydrogenase locus.

**24.6** A researcher has been studying genetic variation in fish populations by using PCR to amplify microsatellite repeats at a particular site on a chromosome (see Chapter 16). The diagram below shows the gel-fractionated products of amplifications with DNA samples from 10 different fish. How many distinct alleles of this microsatellite locus are evident in the gel?



**ANS:** There are four alleles.

**24.7** Within the coding region of a gene, where would you most likely find silent polymorphisms?

**ANS:** In the third position of some of the codons. Due to the degeneracy of the genetic code, different codons can specify the same amino acid. The degeneracy is most pronounced in the third position of many codons, where different nucleotides can be present without changing the amino acid that is specified.

**24.8** Why are the nucleotide sequences of introns more polymorphic than the nucleotide sequences of exons?

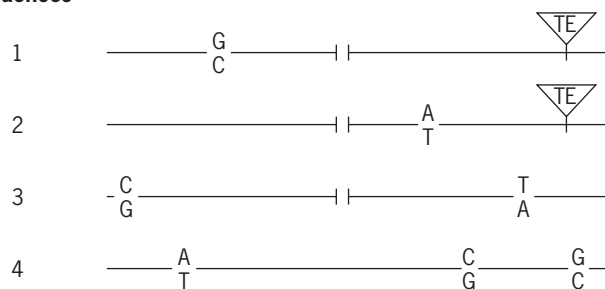
**ANS:** Introns do not encode amino acids; most exons do. Many—perhaps most—of the nucleotides within an intron can be changed without impairing the expression of the gene or the integrity of its polypeptide product. By contrast, many of the nucleotides within exons—especially the first and second positions of codons—are functionally constrained by the amino acids specified.

**24.9** DNA and protein molecules are “documents of evolutionary history.” Why aren’t complex carbohydrate molecules such as starch, cellulose, and glycogen considered “documents of evolutionary history”?

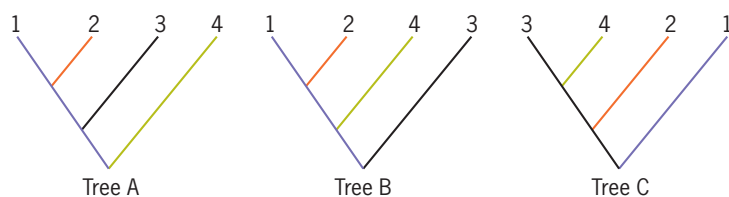
**ANS:** Complex carbohydrates are not “documents of evolutionary history” because, although they are polymers, they are typically made of one subunit incorporated repetitiously into a chain. Such a polymer has little or no “information content.” Thus, there is little or no opportunity to distinguish a complex carbohydrate obtained from two different organisms. Moreover, complex carbohydrates are not part of the genetic machinery; their formation is ultimately specified by the action of enzymes, which are gene products, but they themselves are not genetic material or the products of the genetic material.

**24.10** A geneticist analyzed the sequences of a gene cloned from four different individuals. The four clones were identical except for a few base pair differences, a deletion (gap), and a transposable element (TE) insertion:

#### Sequences



Using this information, compute the minimum number of mutations required to explain the derivation of the four sequences (1, 2, 3, and 4) in the following phylogenetic trees:



Which of these trees provides the most parsimonious explanation for the evolutionary history of the four DNA sequences?

**ANS:** The tree on the right would require nine mutations. These mutations are a deletion in the branch leading to

the common ancestor of sequences 1, 2, and 3; a TE insertion in the branch leading to the common ancestor of sequences 1 and 2; and seven base-pair changes: one leading to sequence 1, another leading to sequence 2, two leading to sequence 3 and three leading to sequence 4. The tree in the middle would also require nine mutations. In this tree, we regard the gap as ancestral—which means that sequence 4 has acquired an “insertion” at the position of the gap. The other mutations are a TE insertion in the branch leading to the common ancestor of sequences 1 and 2, and seven base-pair changes: one leading to sequence 1, another to sequence 2, two leading to sequence 3, and three leading to sequence 4. The tree on the right would also require nine mutations. Here again we regard the gap as ancestral; evidently, an insertion occurred at the position of the gap in the branch leading to sequence 4. The TE insertion can also be regarded as ancestral, with loss occurring in the branch leading to the common ancestor of sequences 3 and 4. There are also seven base-pair changes in this tree: one leading to sequence 1, another leading to sequence 2, two leading to sequence 3 and three leading to sequence 4. These interpretations of the data assume that insertions and deletions (gaps) are reversible and that there is no way of telling which way the sequence evolved—that is, through insertion or deletion. However, if we know, for example, that the TE is a retrotransposon incapable of excision, then we would need more than nine mutations to explain the tree on the right. The TE insertions must have occurred independently in the branches leading to sequences 1 and 2, and there is no need to postulate excision of the TE in the branch leading to the common ancestor of sequences 3 and 4. Thus, on the assumption that the TE is not excisable, 10 mutations are needed to explain the tree on the right. Given this reservation, the left and middle trees provide the most parsimonious explanations for the evolution of the four sequences.

**24.11** The heme group in hemoglobin is held in place by histidines in the globin polypeptides. All vertebrate globins possess these histidines. Explain this observation in terms of the Neutral Theory of Molecular Evolution.

**ANS:** The histidines are rigorously conserved because they perform an important function—anchoring the heme group in hemoglobin. Because these amino acids are strongly constrained by natural selection, they do not evolve by mutation and random genetic drift.

**24.12** During the early evolutionary history of the vertebrates, a primordial globin gene was duplicated to form the  $\alpha$ - and  $\beta$ -globin genes. The rate of evolution of the polypeptides encoded by these duplicate genes has been estimated to be about 0.9 amino acid substitutions per site every billion years. By comparing the human  $\alpha$ - and  $\beta$ -globins, the average number of amino acid substitutions per site has been estimated to be 0.800. From this estimate, calculate when the duplication event that produced the  $\alpha$ - and  $\beta$ -globin genes must have occurred.

- ANS:** The total elapsed evolutionary time is  $0.800/0.9 = 880$  million years, which must be apportioned equally to the  $\alpha$  and  $\beta$  gene lineages by dividing by 2; thus, the time since the duplication event is estimated to be 440 million years.
- 24.13** Ribonuclease, a protein that degrades RNA, is 124 amino acids long. A comparison between the amino acid sequences of cow and rat ribonucleases reveals 40 differences. What is the average number of amino acid substitutions that have occurred per site in these two evolutionary lineages? If the cow and the rat lineages diverged from a common ancestor 80 million years ago, what is the rate of ribonuclease evolution?
- ANS:** Estimate the average number of substitutions per site in the ribonuclease molecule as  $-\ln(S)$ , where  $S = (124 - 40)/124 = 0.68$ , the proportion of amino acids that are the same in the rat and cow molecules. The average number of substitutions per site since the cow and rat lineages diverged from a common ancestor is therefore 0.39. The evolutionary rate in the cow and rat lineages is  $0.39/(2 \times 80 \text{ million years}) = 2.4$  substitutions per site every billion years.
- 24.14** If a randomly mating population is segregating  $n$  selectively neutral alleles of a gene and each allele has the same frequency, what is the frequency of all the homozygotes in the population?
- ANS:** With  $n$  alleles having equal frequency, the frequency of any one allele is  $1/n$ . Under random mating, the frequency of homozygotes for a particular allele is  $(1/n)^2$ . The frequency of all the homozygotes is therefore  $\sum(1/n)^2 = n(1/n)^2 = 1/n$ .
- 24.15** If the evolutionary rate of amino acid substitution in a protein is  $K$ , what is the average length of time between successive amino acid substitutions in this protein?
- ANS:** The reciprocal of the rate, that is,  $1/K$ .
- 24.16** The coding sequence of the alcohol dehydrogenase (*Adb*) gene of *D. melanogaster* consists of 765 nucleotides (255 codons); 192 of these nucleotides are functionally silent—that is, they can be changed without changing an amino acid in the Adh polypeptide. In a study of genetic variation in the *Adb* gene, Martin Kreitman observed that 13 of the 192 silent nucleotides were polymorphic. If the same level of polymorphisms existed among the nonsilent nucleotides of the *Adb* gene, how many amino acid polymorphisms would Kreitman have observed in the populations he studied?
- ANS:** The fraction of polymorphic sites among the silent nucleotides is  $13/192 = 0.068$ . If the same level of polymorphism existed among the nonsilent sites, the number of amino acid polymorphisms would be  $225 \times 0.067 = 17.2$ . Kreitman actually observed only one amino acid polymorphism—evidence that amino acid changes in alcohol dehydrogenase are deleterious.
- 24.17** How might you explain the 1000-fold difference in the evolutionary rates of fibrinopeptide and histone 3?
- ANS:** The protein with the higher evolutionary rate is not as constrained by natural selection as the protein with the lower evolutionary rate.
- 24.18** A geneticist has studied the sequence of a gene in each of three species, A, B, and C. Species A and species B are sister species; species C is more distantly related. The geneticist has calculated the ratio of nonsynonymous (NS) to synonymous (S) nucleotide substitutions in the coding region of the gene in two ways—first, by comparing the gene sequences of species A and C, and second, by comparing the gene sequences of species B and C. The NS:S ratio for the comparison of species B and C is five times greater than it is for the comparison of species A and C. What might this difference in the NS:S ratios suggest?
- ANS:** The difference in the NS:S ratios suggests that in at least one lineage, positive selection has been operating to change nucleotides in the gene.
- 24.19** Dispersed, repetitive sequences such as transposable elements may have played a role in duplicating short regions in a genome. Can you suggest a mechanism? (*Hint:* See Chapter 21 on the Instructor Companion site.)
- ANS:** Repetitive sequences that are near each other can mediate displaced pairing during meiosis. Exchange involving the displaced sequences can duplicate the region between them.
- 24.20** Exon shuffling is a mechanism that combines exons from different sources into a coherent sequence that can encode a composite protein—one that contains peptides from each of the contributing exons. Alternate splicing is a mechanism that allows exons to be deleted during the expression of a gene; the mRNAs produced by alternate splicing may encode different, but related, polypeptides (see Chapter 18). What bearing do these two mechanisms have on the number of genes in a eukaryotic genome? Do these mechanisms help to explain why the gene number in the nematode *Caenorhabditis elegans* is not too different from the gene number in *Homo sapiens*?
- ANS:** Exon shuffling is a way of creating genes that have pieces from disparate sources. Through exon shuffling, the number of genes in a genome could be increased without having to evolve the genes “from scratch.” However, the genome sequencing projects indicate that the gene number in complex, multicellular vertebrates is not much different from the gene number in simple, multicellular invertebrates or in plants. If, judging from their phenotypes, multicellular vertebrates need more gene products than phenotypically simpler invertebrates such as *C. elegans*, alternate splicing could provide some of these gene products without increasing the number of genes.
- 24.21** *Drosophila mauritiana* inhabits the island of Mauritius in the Indian Ocean. *Drosophila simulans*, a close relative, is

widely distributed throughout the world. What experimental tests would you perform to determine if *D. mauritiana* and *D. simulans* are genetically different species?

**ANS:** Cross *D. mauritiana* with *D. simulans* and determine if these two species are reproductively isolated. For instance, can they produce offspring? If they can, are the offspring fertile?

**24.22** Distinguish between allopatric and sympatric modes of speciation.

**ANS:** Allopatric speciation occurs when populations diverge genetically while they are geographically separated. Sympatric speciation occurs when populations diverge genetically while they inhabit the same territory.

**24.23** The *prune* gene (symbol *pn*) is X-linked in *Drosophila melanogaster*. Mutant alleles of this gene cause the eyes to be brown instead of red. A dominant mutant allele of another gene located on a large autosome causes hemizygous or homozygous *pn* flies to die; this dominant mutant allele is therefore called *Killer of prune* (symbol *Kpn*). How could mutants such as these play a role in the evolution of reproductive isolation between populations?

**ANS:** The *Kpn-pn* interaction is an example of the kind of negative epistasis that might prevent populations that have evolved separately from merging into one panmictic population. The *Kpn* mutation would have evolved in one population and the *pn* mutation in another, geographically separate population. When the populations merge, the two mutations can be brought into the same

fly by interbreeding. If the combination of these mutations is lethal, then the previously separate populations will not be able to exchange genes, that is, they will be reproductively isolated.

**24.24** A segment of DNA in an individual may differ at several nucleotide positions from a corresponding DNA segment in another individual. For instance, one individual may have the sequence ...A...G...C... and another individual may have the sequence ...T...A...A.... These two DNA segments differ in three nucleotide positions. Because the nucleotides within each segment are tightly linked, they will tend to be inherited together as a unit, that is, without being scrambled by recombination. We call such heritable units DNA haplotypes. Through sampling and DNA sequencing, researchers can determine which DNA haplotypes are present in a particular population. When this kind of analysis is performed on human populations by sequencing, for example, a segment of mitochondrial DNA, it is found that samples from Africa exhibit more haplotype diversity than samples from other continents. What does this observation tell us about human evolution?

**ANS:** More haplotype diversity refers to the number of different haplotypes that are found in a population. If African populations of humans have the greatest haplotype diversity, then these populations appear to have had a longer time to accumulate different haplotypes—that is, they are older than other populations. Greater haplotype diversity in African populations of humans is therefore evidence that African populations were at the root of the modern human evolutionary tree.