

5

SEX DETERMINATION, SEX LINKAGE, AND PEDIGREE ANALYSIS

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3. To analyze the inheritance patterns of traits that loci on the sex chromosomes control 95
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Three generations of a family.

(© Frank Siteman/Tony Stone Images.)

We ended chapter 3 with a discussion of the chromosomal theory of heredity, stated lucidly in 1903 by Walter Sutton, that genes are located on chromosomes. In 1910, T. H. Morgan, a 1933 Nobel laureate, published a paper on the inheritance of white eyes in fruit flies. The mode of inheritance for this trait, discussed later in this chapter, led inevitably to the conclusion that the locus for this gene is on a chromosome that determines the sex of the flies: when a white-eyed male was mated with a red-eyed female, half of the F_2 sons were white-eyed and half were red-eyed; all F_2 daughters were red-eyed. Not only was this the first evidence that localized a particular gene to a particular chromosome, but this study also laid the foundation for our understanding of the genetic control of sex determination.

SEX DETERMINATION

Patterns

At the outset, we should note that the sex of an organism usually depends on a very complicated series of developmental changes under genetic and hormonal control. However, often one or a few genes can determine which pathway of development an organism takes. Those switch genes are located on the **sex chromosomes**, a heteromorphic pair of chromosomes, when those chromosomes exist.

However, sex chromosomes are not the only determinants of an organism's sex. The ploidy of an individual, as in many hymenoptera (bees, ants, wasps), can determine sex; males are haploid and females are diploid. Allelic mechanisms may determine sex by a single allele or multiple alleles not associated with heteromorphic chromosomes; even environmental factors may control sex. For example, temperature determines the sex of some geckos, and the sex of some marine worms and gastropods depends on the substrate on which they land. In this chapter, however, we concentrate on chromosomal sex-determining mechanisms.

Sex Chromosomes

Basically, four types of chromosomal sex-determining mechanisms exist: the XY, ZW, X0, and compound chromosomal mechanisms. In the XY case, as in human beings or fruit flies, the females have a homomorphic pair of chromosomes (XX) and males are heteromorphic (XY). In the ZW case, males are homomorphic (ZZ), and females are heteromorphic (ZW). (XY and ZW are chromosome notations and imply nothing about the sizes or shapes of these chromosomes.) In the X0 case, the organism has only one

sex chromosome, as in some grasshoppers and beetles; females are usually XX and males X0. And in the compound chromosome case, several X and Y chromosomes combine to determine sex, as in bedbugs and some beetles. We need to emphasize that the chromosomes themselves do not determine sex, but the genes they carry do. In general, the genotype determines the type of gonad, which then determines the phenotype of the organism through male or female hormonal production.

The XY System

The XY situation occurs in human beings, in which females have forty-six chromosomes arranged in twenty-three homologous, homomorphic pairs. Males, with the same number of chromosomes, have twenty-two homomorphic pairs and one heteromorphic pair, the XY pair (fig. 5.1). During meiosis, females produce gametes that contain only the X chromosome, whereas males produce two kinds of gametes, X- and Y-bearing (fig. 5.2). For this reason, females are referred to as **homogametic** and males as **heterogametic**. As you can see from figure 5.2, in people, fertilization has an equal chance of producing either male or female offspring. In *Drosophila*, the system is the same, but the Y chromosome is almost 20% larger than the X chromosome (fig. 5.3).

Since both human and *Drosophila* females normally have two X chromosomes, and males have an X and a Y chromosome, it seems impossible to know whether maleness is determined by the presence of a Y chromosome or the absence of a second X chromosome. One way to resolve this problem would be to isolate individuals with odd numbers of chromosomes. In chapter 8, we examine the causes and outcomes of anomalous chromosome numbers. Here, we consider two facts from that chapter. First, in rare instances, individuals form, although they are not necessarily viable, with extra sets of chromosomes. These individuals are referred to as **polyploids** (*triploids* with $3n$, *tetraploids* with $4n$, etc.). Second, also infrequently, individuals form that have more or fewer than the normal number of any one chromosome. These **aneuploids** usually come about when a pair of chromosomes fails to separate properly during meiosis, an occurrence called **nondisjunction**. The existence of polyploid and aneuploid individuals makes it possible to test whether the Y chromosome is male determining. For example, a person or a fruit fly that has all the proper nonsex chromosomes, or **autosomes** (forty-four in human beings, six in *Drosophila*), but only a single X without a Y would answer our question. If the Y were absolutely male determining, then this X0 individual should be female. However, if the sex-determining mechanism is a result of the number of X chromosomes, this individual should be a male. As it turns out, an X0 individual is a *Drosophila* male and a human female.

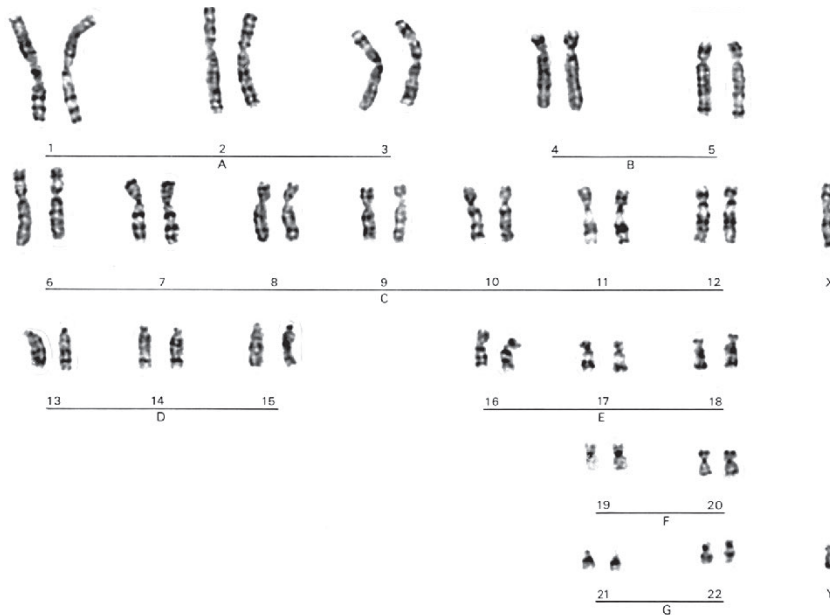
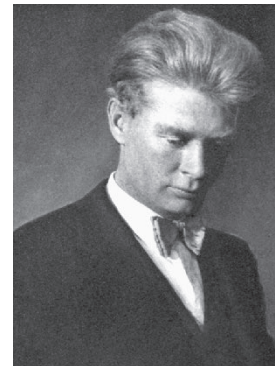


Figure 5.1 Human male karyotype. Note the X and Y chromosomes. A female would have a second X chromosome in place of the Y. (Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

Genic Balance in *Drosophila*

When geneticist Calvin Bridges, working with *Drosophila*, crossed a triploid ($3n$) female with a normal male, he observed many combinations of autosomes and sex chromosomes in the offspring. From his results, Bridges suggested in 1921 that sex in *Drosophila* is determined by the balance between (ratio of) autosomal alleles that favor maleness and alleles on the X chromosomes that favor femaleness. He calculated a ratio of X chromosomes to autosomal sets to see if this ratio would predict the sex of a fly. An **autosomal set (A)** in *Drosophila* consists of one chromosome from each autosomal pair, or three chromosomes. (An autosomal set in human beings consists of twenty-two chromosomes.) Table 5.1, which presents his results, shows that Bridges's **genic balance**



Calvin B. Bridges (1889–1938). (From *Genetics* 25 (1940): frontispiece. Courtesy of the Genetics Society of America.)

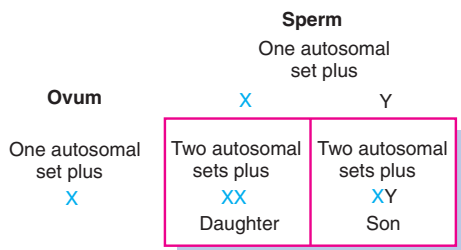


Figure 5.2 Segregation of human sex chromosomes during meiosis, with subsequent zygote formation.

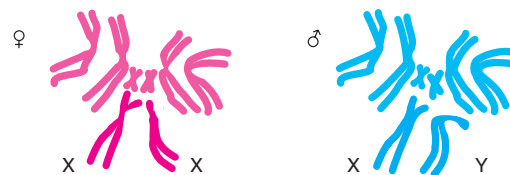


Figure 5.3 Chromosomes of *Drosophila melanogaster*.

Table 5.1 Data Supporting Bridges's Theory of Sex Determination by Genic Balance in *Drosophila*

Number of X Chromosomes	Number of Autosomal Sets (A)	Total Number of Chromosomes	$\frac{X}{A}$ Ratio	Sex
3	2	9	1.50	Metafemale
4	3	13	1.33	Female
4	4	16	1.00	Female
3	3	12	1.00	Female
2	2	8	1.00	Female
1	1	4	1.00	Female
2	3	11	0.67	Intersex
1	2	7	0.50	Male
1	3	10	0.33	Metamale

theory of sex determination was essentially correct. When the X:A ratio is 1.00, as in a normal female, or greater than 1.00, the organism is a female. When this ratio is 0.50, as in a normal male, or less than 0.50, the organism is a male. At 0.67, the organism is an **intersex**. **Metamales** (X/A = 0.33) and **metafemales** (X/A = 1.50) are usually very weak and sterile. The metafemales usually do not even emerge from their pupal cases.

A **sex-switch** gene has been discovered that directs female development. This gene, **Sex-lethal** (*Sxl*), is located on the X chromosome. (It was originally called *female-lethal* because mutations of this gene killed female embryos.) Apparently, *Sxl* has two states of activity. When it is "on," it directs female development; when it is "off," maleness ensues. Other genes located on the X chromosome and the autosomes regulate this sex-switch gene. Genes on the X chromosome that act to regulate *Sxl* into the on state (female development) are called **numerator elements** because they act on the numerator of the X/A genic balance equation. Genes on the autosomes that act to regulate *Sxl* into the off state (male development) are called **denominator elements**. Geneticists have discovered four numerator elements—genes named *sisterless-a*, *sisterless-b*, *sisterless-c*, and *runt*. *Sxl* "counts" the number of X chromosomes; it turns on when two are present. It counts by measuring the level of the numerator genes' protein product. If the level is high, *Sxl* turns on, and the organism develops as a female. If the level is relatively low, *Sxl* does not turn on, and development proceeds as a male.

Sex Determination in Human Beings

Since the X0 genotype in human beings is a female (having Turner syndrome), it seems reasonable to conclude that the Y chromosome is male determining in human beings. The fact that persons with Klinefelter syndrome (XXY, XXXY, XXXXY) are all male, and XXX,

XXXX, and other multiple-X karyotypes are all female, verifies this idea. (More details on these anomalies are presented in chapter 8.) For a long time, researchers have sought a single gene, a **testis-determining factor (TDF)**, located on the Y chromosome that acts as a sex switch to initiate male development. Human embryologists had discovered that during the first month of embryonic development, the gonads that develop are neither testes nor ovaries, but instead are indeterminate. At about six or seven weeks of development, the indeterminate gonads become either ovaries or testes.

In the 1950s, Ernst Eichwald found that males had a protein on their cell surfaces not found in females; he discovered that female mice rejected skin grafts from genetically identical brothers, whereas the brothers accepted grafts from sisters. This implies that an antigen exists on the surface of male cells that is not found on female cells. This protein was called the *histocompatibility Y antigen (H-Y antigen)*. The gene for this protein was found on the Y chromosome, near the centromere. At first, scientists believed it to be the sex switch: if the gene were present, the gonads would begin development as testes. Further male development, as in male secondary sexual characteristics, came about through the testosterone the functional testes produced. If the gene were absent, the gonads would develop into ovaries. Recently, however, by studying "sex-reversed" individuals, biologists refuted this theory.

Sex-reversed individuals are XX males or XY females. David Page, at the Whitehead Institute for Biomedical Research, found twenty XX males who had a small piece of the short arm of the Y chromosome attached to one of their X chromosomes. He found six XY females in whom the Y chromosome was missing the same small piece at the end of its short arm. This region, which did not contain the *HYA* gene, must carry the testis-determining factor. The first candidate gene from this region believed to code for the testis-determining factor was named the



David Page (1956–).
(Courtesy of Dr. David Page.)



Figure 5.4 Normal male mouse (*left*) and female littermate given the *Sry* gene (*right*). Both mice are indistinguishably male.
(Courtesy of Robin Lovell-Badge.)

ZFY gene, for zinc finger on the Y chromosome. Zinc fingers are protein configurations known to interact with DNA (discussed in detail in chapter 16). Thus, researchers believed that the *ZFY* gene, coding for the testis-determining factor, worked by directly interacting with DNA. (Later in the book we look at the way regulatory genes, whose proteins interact with DNA, work.) However, men who lack the *ZFY* gene have been found, suggesting that the testis-determining factor is very close to, but not, the *ZFY* gene. From work in mice, it has been suggested that the *ZFY* gene controls the initiation of sperm cell development, but not maleness.

In 1991, Robin Lovell-Badge and Peter Goodfellow and their colleagues in England isolated a gene called **Sex-determining region Y (*SRY*)**—*Sry* in mice—adjacent to the *ZFY* gene. *Sry* has been positively identified as the testis-determining factor because, when injected into normal (XX) female mice, it caused them to develop as males (fig. 5.4). Although these XX males are sterile, they appear as normal males in every other way. (We discuss in chapter 13 how scientists introduce new genes into an organism.) Note also that the mouse and human systems are very similar genetically, and the homologous genes have been isolated from both. However, at present,

the human *SRY* gene does not convert XX female mice into males. Like the *ZFY* gene product, Sry protein (the protein the *SRY* gene produces) also binds to DNA.

The Sry protein appears to bind to at least two genes. One, the p450 aromatase gene, has a protein product that converts the male hormone testosterone to the female hormone estradiol; the Sry protein inhibits production of p450 aromatase. The second gene the Sry protein affects is the gene for the Müllerian-inhibiting substance, which induces testicular development and the digression of female reproductive ducts; the Sry protein enhances this gene's activity. Thus, the Sry protein points an indifferent embryo toward maleness and the maintenance of testosterone production. The sex switch initiates a developmental sequence involving numerous genes. Eva Eicher and Linda Washburn have developed a model in which two pathways of coordinated gene action help determine sex, one pathway for each sex. The first gene in the ovary-determining pathway is termed *ovary determining (Od)*. The first gene in the testis-determining pathway must function before the *Od* gene begins, in order to allow XY individuals to develop as males. Once the steps of a pathway are initiated, the other pathway is inhibited (fig. 5.5).



Robin Lovell-Badge (1953–).
(Courtesy of Robin Lovell-Badge.)



Peter Goodfellow (1951–).
(Courtesy of Peter Goodfellow.)

Other Chromosomal Systems

The X0 system, sometimes referred to as an X0-XX system, occurs in many species of insects. It functions just as the XY chromosomal mechanism does, except that instead of a Y chromosome, the heterogametic sex (male) has only one X chromosome. Males produce gametes that contain either an X chromosome or no sex chromosome, whereas all the gametes from a female contain the X chromosome. The result of this arrangement is that females have an even number of chromosomes (all in homomorphic pairs) and males have an odd number of chromosomes.

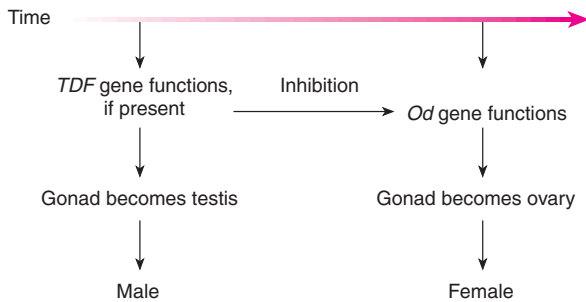


Figure 5.5 A model for the initiation of gonad determination in mammals.

The ZW system is identical to the XY system except that males are homogametic and females are heterogametic. This situation occurs in birds, some fishes, and moths.

Compound chromosomal systems tend to be complex. For example, *Ascaris incurva*, a nematode, has eight X chromosomes and one Y. The species has twenty-six autosomes. Males have thirty-five chromosomes ($26A + 8X + Y$), and females have forty-two chromosomes ($26A + 16X$). During meiosis, the X chromosomes unite end to end and so behave as one unit.

The Y Chromosome

In both human beings and fruit flies, the Y chromosome has very few functioning genes. In human beings, two homologous regions exist, one at either end of the X and Y chromosomes, allowing the chromosomes to pair during meiosis. These regions are termed **pseudoautosomal**. Mapping the Y chromosome (see chapters 6 and 13) has shown us the existence of about thirty-five genes (fig. 5.6). Other, nonfunctioning genes are present, too, remnants of a time in the evolutionary past when those genes were probably active (box 5.1). The *Drosophila* Y chromosome is known to carry genes for at least six fertility factors, two on the short arm (*ks-1* and *ks-2*) and four on the long arm (*kl-1*, *kl-2*, *kl-3*, and *kl-5*). The Y chromosome carries two other known genes: *bobbed*, which is a locus of ribosomal RNA genes (the nucleolar organizer), and *Suppressor of Stellate* or *Su(Ste)*, a gene required for RNA splicing (see chapter 10). The fertility factors code for proteins needed during spermatogenesis. For example, *kl-5* codes for part of the dynein motor needed for sperm flagellar movement.

Sex Determination in Flowering Plants

Flowering plant species (angiosperms) generally have three kinds of flowers: hermaphroditic, male, and female.

Hermaphroditic flowers have both male and female parts. The male parts are the anthers and filaments, making up the *stamen*, and the female parts are the stigma, style, and ovary, making up the *pistil* (see fig. 2.2). Ninety percent of angiosperms have hermaphroditic flowers. Of the 10% of the species that have unisexual flowers, some are *monoecious* (Greek, one house), bearing both male and female flowers on the same plant (e.g., walnut); and some are *dioecious* (Greek, two houses), having plants with just male or just female flowers (e.g., date palm).

Within the group of plant species with unisexual flowers, sex-determining mechanisms vary. Some species have a single locus determining sex, some have two or more loci involved in sex determination, and some have X and Y chromosomes. In most of the species with X and Y chromosomes, the sex chromosomes are indistinguishable. Among these species, most have heterogametic males, although in some species, such as the strawberry, females are heterogametic. In the very few species that have distinguishable X and Y chromosomes—only thirteen are known—two sex-determination mechanisms are found. One is similar to the system in mammals, in which the Y chromosome has a gene or genes present

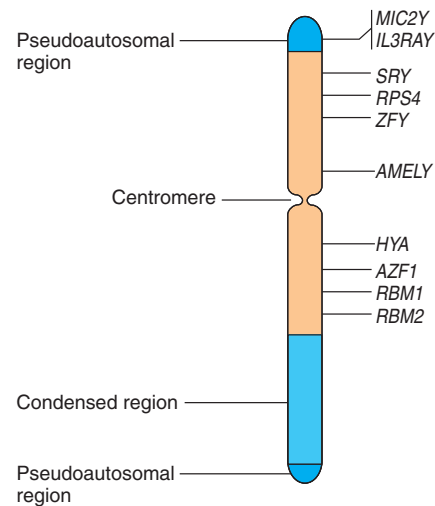


Figure 5.6 The human Y chromosome. In addition to the genes shown, the Y chromosome carries other genes, homologous to X chromosome genes, that do not function because of accumulated mutations. Some of these are in multiple copies. Note the two pseudoautosomal regions that allow synapsis between the Y and X chromosomes. The gene symbols shown include *MIC2Y*, T cell adhesion antigen; *IL3RAY*, interleukin-3 receptor; *RPS4*, a ribosomal protein; *AMELY*, amelogenin; *HYA*, histocompatibility Y antigen; *AZF1*, azoospermia factor 1 (mutants result in tailless sperm); and *RBM1*, *RBM2*, RNA binding proteins 1 and 2. (Adapted from Online Mendelian Inheritance in Man website. <http://www3.ncbi.nlm.nih.gov/omim/>. Reprinted with permission.)

BOX 5.1

Evolutionary biologists have asked, Why does sex exist? A haploid, asexual way of life seems like a very efficient form of existence. Haploid fungi can produce thousands of haploid spores, each of which can grow into a new colony. What evolutionary benefit do organisms gain by developing diploidy and sexual processes? Although this may not seem like a serious question, evolutionary biologists look for compelling answers.

In chapter 21, we discuss evolutionary thinking in some detail. For the moment, accept that evolutionary biologists look for an adaptive advantage in most evolutionary outcomes. Thus they ask, What is better about the combining of gametes to produce a new generation of offspring? Why would a diploid organism take a random sample of its genome and combine it with a random sample of someone else's genome to produce offspring? Why not simply produce offspring by mitosis? If offspring are produced by mitosis, all of an individual's genes pass into the next generation with every offspring. Not only does just half the genome of an individual pass into the next generation with every offspring produced sexually, but that half is a random jumble of what might be a very highly adapted genome. In addition, males are doubly expensive to produce because males themselves do not produce offspring: males fertilize females who produce offspring. Thus, on the surface, evolutionary biologists need to find very strong reasons for an organism to turn to sexual reproduction when an individual might be at an advantage evolutionarily to reproduce asexually.

There have been numerous suggestions as to the advantage of sex, nicely summarized in a 1994 article by James Crow, of the University of

Experimental Methods

Why Sex and Why Y?

Wisconsin, in *Developmental Genetics*, and more recently in a special section of the 25 September 1998 issue of *Science* magazine. We aren't really sure what the true evolutionary reasons for sex are, but at least three explanations seem reasonable to evolutionary biologists:

- *Adjusting to a changing environment.* Sexual reproduction allows for much more variation in organisms. A haploid, asexual organism collects variation over time by mutation. A sexual organism, on the other hand, can achieve a tremendous amount of variation by recombination and fertilization. Remember that a human being can produce potentially $2^{100,000}$ different gametes. In a changing environment, a sexually reproduced organism is much more likely than an asexual organism to produce offspring that will be adapted to the changes.
- *Combining beneficial mutations.* As mentioned, a haploid, asexual organism accrues mutations as they happen over time in a given individual. A sexual organism can combine beneficial mutations each generation by recombination and fertilization. Thus, sexually reproducing organisms can adapt at a much more rapid rate than asexual organisms.
- *Removing deleterious mutations.* Mutation is more likely to produce deleterious changes

than beneficial ones. An asexual organism gathers more and more deleterious mutations as time goes by (a process referred to as *Muller's ratchet*, in honor of Nobel Prize-winning geneticist H. J. Muller and referring to a ratchet wheel that can only go forward). Sexually reproducing organisms can eliminate deleterious mutations each generation by forming recombined offspring that are relatively free of mutation.

Hence, this list provides three of the generally assumed advantages of sexual reproduction that offset its disadvantages.

Another subtle question about sexual reproduction that evolutionary biologists ask is, Why is there a Y chromosome? In other words, why do we have, in some species (e.g., people), a heteromorphic pair of chromosomes involved in sex determination, with one of the chromosomes having the gene for that sex and very few other loci? In people, the Y chromosome is basically a degenerate chromosome with very few loci. This morphological difference between the members of the sex chromosome pair is puzzling. After all, chromosome pairs that do not carry sex-determining loci do not tend to be morphologically heterogeneous. Consider the following possible scenario that Virginia Morell presented in the 14 January 1994 issue of *Science*.

In a particular species in the past—evolutionarily speaking—a sex-determining gene arises on a particular chromosome. One allele at this locus confers maleness on its bearer. The absence of this allele causes the carrier to be female. At this point, millions of years ago, the sex chromosomes are not morphologically heterogeneous: the X and Y chromosomes are identical. In time,

however, the Y chromosome comes to carry a gene that is beneficial to the male but not the female. For example, there might be a gene with an allele for a colorful marking; this allele confers a reproductive advantage for the male but also confers a predatory risk on the bearer, whether male or female. Males have a reproductive advantage to outweigh the predation risk, whereas females have none. Thus, the allele is favored in males and selected against in females.

An evolutionary solution to this situation is to isolate the gene for this marking on the Y chromosome and keep it off the X chromosome so that males have it but females do not. This can take place if the two chromosomes do not recombine over most of their lengths. Assume then, that some mechanism evolves to prevent recombination of the X and Y chromosomes. Thereafter, the Y chromosome degenerates, losing most of its genes but retaining the sex-determining locus and the loci conferring an advantage on males but a disadvantage on females.

What evidence do we have that any of these links in this complex line of logic are true? To begin with, when we look at evolutionary lineages, we usually see a spectrum of species

with sex chromosomes in all stages of differentiation. Evolutionary biologists generally accept the notion that the similar sex chromosomes are the original condition and the morphologically heterogeneous sex chromosomes are the more evolved condition. In addition, as reported in the same issue of *Science*, William Rice of the University of California at Santa Cruz has shown experimentally with fruit flies that if recombination is prevented between sex chromosomes, the Y chromosome degenerates; it loses the function of many loci that are also found on the X chromosome. Rice showed this with an ingenious set of experiments that successfully prevented a nascent Y chromosome from recombining with the X. The results confirmed the prediction that the Y chromosome degenerates (fig. 1).

More recently, in an October 1999 article in *Science*, Bruce Lahn and David Page, at the Massachusetts Institute of Technology, reported research findings indicating that degeneration of the human Y chromosome has taken place in four stages, starting as long as 320 million years ago in our mammalian ancestors. Using DNA sequence data and methods discussed in chapter 21, they showed

that the 19 genes known from both the X and Y chromosomes are arranged as if the Y chromosome has undergone four rearrangements, each preventing further recombination of the X and Y. According to their calculations, this process began shortly after the mammals split from the birds, which themselves went on to evolve a ZW pair of sex chromosomes.

Clearly, much more work is needed to validate all the steps in this logical, evolutionary argument. However, at this point, enough empirical support exists to make the idea attractive to evolutionary biologists.

Although we have gotten a bit ahead of ourselves by talking about subtle evolutionary arguments before reaching that material in the book, it is a good idea to keep an evolutionary perspective on processes and structures. Presumably, evolution has shaped us and the biological world in which we live. If that is so, we should be able to figure out how evolution was working. That thinking should hold from the level of the molecule (e.g., enzymes and DNA) to that of the whole organism. Behind every process and structure should be a hint of the evolutionary pressures that caused that structure or process to evolve.

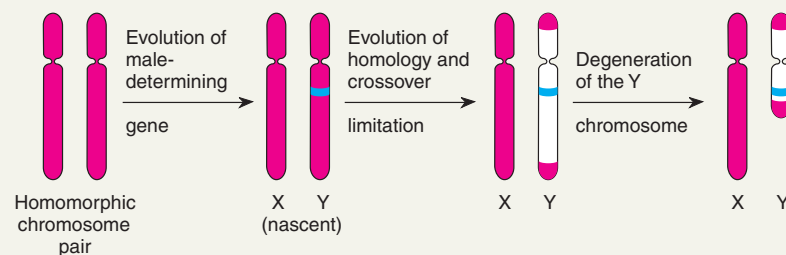


Figure 1 Evolution of a hypothetical Y chromosome. Red represents homologous regions, blue shows the male-determining gene, and white marks evolved areas of the Y chromosome that no longer recombine with the X chromosome.

that actively determine male-flowering plants. The other system is similar to that found in fruit flies, in which the X:A ratio determines sex.

In the mammalian-type system, the Y chromosome carries genes needed for the development of male flower parts while suppressing the development of female parts. An example of this is in the white campion (*Silene latifolia*). In the *Drosophila*-type system, found in the sorrel (*Rumex acetosa*), the ratios determine sex exactly as in the flies. That is, an X:A ratio of 0.5 or lower results in a male; a ratio of 1.0 or higher results in a female; and an intermediate ratio results in a plant with hermaphroditic flowers. It seems that all flowers have the potential to be hermaphroditic. That is, flower primordia for hermaphroditic, male, and female flowers look identical during early development. The simplest mechanism of sex determination would involve repressing the development of the female flower parts in male flowers and repressing the male flower parts in female flowers. Current research indicates that this repression of one component or another is probably involved in most flower sex determination and is under genetic and hormonal control. (We discuss further the genetic control of flower development in chapter 16.)

DOSAGE COMPENSATION

In the XY chromosomal system of sex determination, males have only one X chromosome, whereas females have two. Thus, disregarding pseudoautosomal regions, males have half the number of X-linked alleles as females for genes that are not primarily related to gender. A question arises: How does the organism compensate for this dosage difference between the sexes, given the potential for serious abnormality? In general, an incorrect number of autosomes is usually highly deleterious to an organism (see chapter 8). In human beings and other mammals, the necessary **dosage compensation** is accomplished by the inactivation of one of the X chromosomes in females so that both males and females have only one functional X chromosome per cell.

In 1949, M. Barr and E. Bertram first observed a condensed body in the nucleus that was not the nucleolus. Noting that normal female cats show a single condensed body, while males show none, these researchers referred to the body as sex chromatin, since known as a **Barr body** (fig 5.7). Mary Lyon then suggested that this Barr body represented an inactivated X chromosome, which in females becomes tightly coiled into heterochromatin, a condensed, and therefore visible, form of chromatin.

Various lines of evidence support the **Lyon hypothesis** that only one X chromosome is active in any cell. First, XXY males have a Barr body, whereas XO females have none. Second, persons with abnormal numbers of X



Mary F. Lyon (1925–).
(Courtesy of Dr. Mary F. Lyon.)

chromosomes have one fewer Barr body than they have X chromosomes per cell: XXX females have two Barr bodies and XXXX females have three.

Proof of the Lyon Hypothesis

Direct proof of the Lyon hypothesis came when cytologists identified the Barr body in normal females as an X chromosome. Genetic evidence also supports the Lyon hypothesis: Females heterozygous for a locus on the X chromosome show a unique pattern of phenotypic expression. We now know that in human females, an X chromosome is inactivated in each cell on about the twelfth day of embryonic life; we also know that the inactivated X is randomly determined in a given cell. From that point on, the same X remains a Barr body for future cell generations. Thus, heterozygous females show **mosaicism** at the cellular level for X-linked traits. Instead of being typically heterozygous, they express only one or the other of the X chromosomal alleles in each cell.

Glucose-6-phosphate dehydrogenase (G-6-PD) is an enzyme that a locus on the X chromosome controls. The

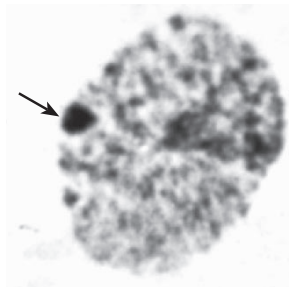


Figure 5.7 Barr body (arrow) in the nucleus of a cheek mucosal cell of a normal woman. This visible mass of heterochromatin is an inactivated X chromosome. (Thomas G. Brewster and Park S. Gerald, "Chromosome disorders associated with mental retardation," *Pediatric Annals*, 7, no. 2, 1978. Reproduced courtesy of Dr. Thomas G. Brewster, Foundation for Blood Research, Scarborough, Maine.)

enzyme occurs in several different allelic forms that differ by single amino acids. Thus, both forms (A and B) will dehydrogenate glucose-6-phosphate—both are fully functional enzymes—but because they differ by an amino acid, they can be distinguished by their rate of migration in an electrical field (one form moves faster than another). This electrical separation, termed **electrophoresis**, is carried out by placing samples of the enzymes in a supporting gel, usually starch, polyacrylamide, agarose, or cellulose acetate (fig. 5.8 and box 5.2). After the electric current is applied for several hours, the enzymes move in the gel as bands, revealing the distance each enzyme traveled. Since blood serum is a conglomerate of proteins from many cells, the serum of a female heterozygote (fig. 5.8, lane 3) has both A and B forms (bands), whereas any single cell (lanes 4–10) has only one or the other. Since the gene for glucose-6-phosphate dehydrogenase is carried on the X chromosome, this electrophoretic display indicates that only one X is active in any particular cell.

Another aspect of the glucose-6-phosphate dehydrogenase system provides further proof of the Lyon hypothesis. If a cell has both alleles functioning, both A and B proteins should be present. Since the functioning glucose-6-phosphate dehydrogenase enzyme is a dimer (made up of two protein subunits), 50% of the enzymes should be heterodimers (AB). These would form a third, intermediate band between the A form (AA dimer) and the B form (BB dimer; fig. 5.9). The lack of heterodimers in the blood of heterozygotes is further proof that both G-6-PD alleles are not active within the same cells. That is, in any one cell, only AA or BB dimers can form, because no single cell has both the A and B forms.

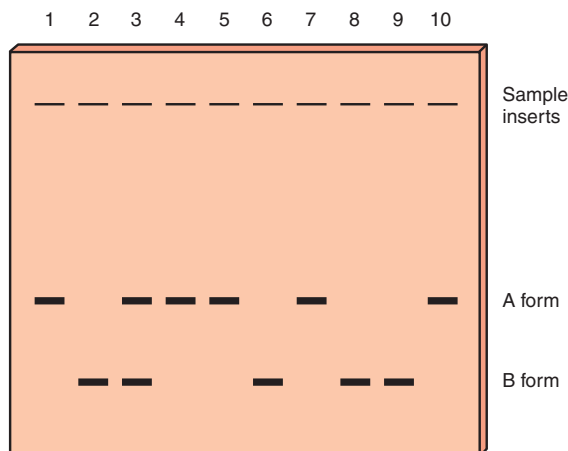


Figure 5.8 Electrophoretic gel stained for glucose-6-phosphate dehydrogenase. Lanes 1–3 contain blood from an AA homozygote, a BB homozygote, and an AB heterozygote, respectively. Lanes 4–10 contain homogenates of individual cells of an AB heterozygote.

The Lyon hypothesis has been demonstrated with many X-linked loci, but the most striking examples are those for color phenotypes in some mammals. For example, the tortoiseshell pattern of cats is due to the inactivation of X chromosomes (fig. 5.10). Tortoiseshell cats are normally females heterozygous for the *yellow* and *black* alleles of the X-linked color locus. They exhibit patches of these two colors, indicating that at a certain stage in development, one or the other of the X chromosomes was inactivated and all of the ensuing daughter cells in that line kept the same X chromosome inactive. The result is patches of coat color.

The X chromosome is inactivated starting at a point called the **X inactivation center (XIC)**. That region contains a gene called *XIST* (for *X inactive-specific transcripts*, referring to the transcriptional activity of this gene in the inactivated X chromosome). The *XIST* gene has been putatively identified as the gene that initiates the inactivation of the X chromosome. This gene is known to be active only in the inactive X chromosome in a normal XX female. Another aspect of “Lyonization” is that several other loci are known to be active on the inactivated X chromosome; they are active in both X chromosomes, even though one is heterochromatic (inactivated). Although several of these loci are in the pseudoautosomal region of the short arm of the X chromosome, several other of the thirty or more genes known to be active are on other places on the mammalian X chromosome. Active genes on the inactive X include the gene for the enzyme steroid sulphatase; the red-cell antigen *Xg^a*; *MIC2*; a *ZFY*-like gene termed *ZFX*; the gene for Kallmann syndrome; and several others.

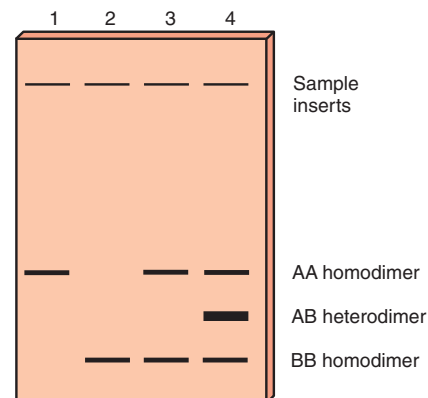


Figure 5.9 Electrophoretic gel stained for glucose-6-phosphate dehydrogenase. Lanes 1 and 2 contain blood serum from AA and BB women, respectively, and lane 3 contains serum from an AB heterozygote. Lane 4 shows the pattern expected if both the A and B alleles were active within the same cell.

BOX 5.2

Electrophoresis, a technique for separating relatively similar types of molecules (for example, proteins and nucleic acids), has opened up new and exciting areas of research in population, biochemical, and molecular genetics. It has allowed us to see variations in large numbers of loci, previously difficult or impossible to sample. In biochemical genetics, electrophoretic techniques can be used to study enzyme pathways. In molecular genetics,

Experimental Methods

Electrophoresis



electrophoresis is used to sequence nucleotides (see chapter 13) and to assign various loci to particular chro-

somes. In population genetics (see chapter 21), electrophoresis has made it possible to estimate the amount of variability that occurs in natural populations.

Here we discuss protein electrophoresis, a process that entails placing a sample—often blood serum or a cell homogenate—at the top of a gel prepared from a suitable substrate (e.g., hydrolyzed starch, polyacrylamide, or cellulose acetate) and a buffer. An electrical current is

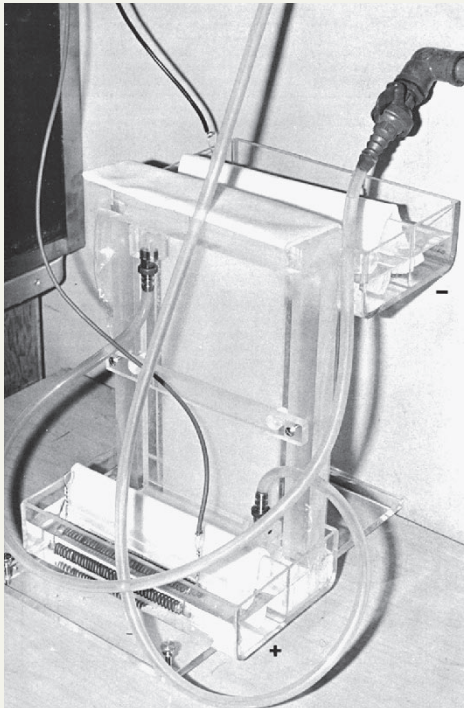


Figure 1 Vertical starch gel apparatus. Current flows from the upper buffer chamber to the lower one by way of the paper wicks and the starch gel. Cooling water flows around the system.

(R. P. Canham, "Serum protein variations and selection in fluctuating populations of cricetid rodents," Ph.D. thesis, University of Alberta, 1969. Reproduced by permission.)

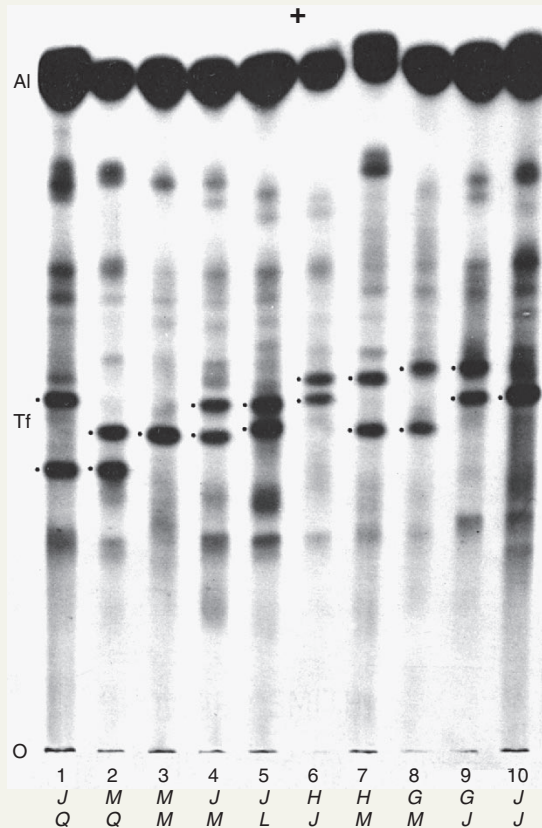
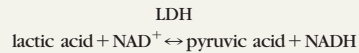


Figure 2 Ten samples of deer mouse (*Peromyscus maniculatus*) blood studied for general protein. *Al* is albumin and *Tf* is transferrin, the two most abundant proteins in mammalian blood. The six *Tf* allozymes are labeled G, H, J, L, M, and Q. (R. P. Canham, "Serum protein variations and selection in fluctuating populations of cricetid rodents," Ph.D. thesis, University of Alberta, 1969. Reproduced by permission.)

passed through the gel to cause charged molecules to move (fig. 1), and the gel is then treated with a dye that stains the protein. In the simplest case, if a protein is homogeneous (usually the product of a homozygote), it forms a single band on the gel. If it is heterogeneous (usually the product of a heterozygote), it forms two bands. This is because the two allelic protein products differ by an amino acid; they have different electrical charges and therefore travel through the gel at different rates (see fig. 5.8). The term **allozyme** refers to different electrophoretic forms of an enzyme controlled by alleles at the same locus.

Figure 2 shows samples of mouse blood serum that have been stained for protein. Most of the staining reveals albumins and β -globulins (transferrin). Because they are present in very small concentrations, many enzymes present in the serum are not visible, but a stain that is specific for a particular enzyme can make that enzyme visible on the gel.

For example, lactate dehydrogenase (LDH) can be located because it catalyzes this reaction:



Thus, we can stain specifically for the lactate dehydrogenase enzyme by adding the substrates of the enzyme (lactic acid and nicotinamide adenine dinucleotide, NAD^+) and a suitable stain specific for a product of the enzyme reaction (pyruvic acid or nicotinamide adenine dinucleotide, reduced form, NADH). That is, if lactic acid and NAD^+ are poured on the gel, only lactate dehydrogenase converts them to pyruvic acid and NADH . We can then test for the presence of NADH by having it reduce the dye, nitro blue tetrazolium, to the blue precipitate, formazan, an electron carrier. We then add all the preceding reagents and look for blue bands on the gel (fig. 3).

In addition to its uses in population genetics and chromosome mapping, electrophoresis has been ex-

tremely useful in determining the structure of many proteins and for studying developmental pathways. As we can see from the lactate dehydrogenase gel in figure 3, five bands can occur. In some tissues of a homozygote, these bands occur roughly in a ratio of 1:4:6:4:1. This pattern can come about if the enzyme is a tetramer whose four subunits are random mixtures of two gene products (from the *A* and *B* loci). Thus we would get

AAAA (1/16)
AAAB (4/16)
AABB (6/16)
ABBB (4/16)
BBBB (1/16)

(Note that the ratio 1:4:6:4:1 is the expansion of $[A + B]^4$, and the relative "intensity" of each band—the number of protein doses—is calculated from the rule of unordered events described in chapter 4.)

continued

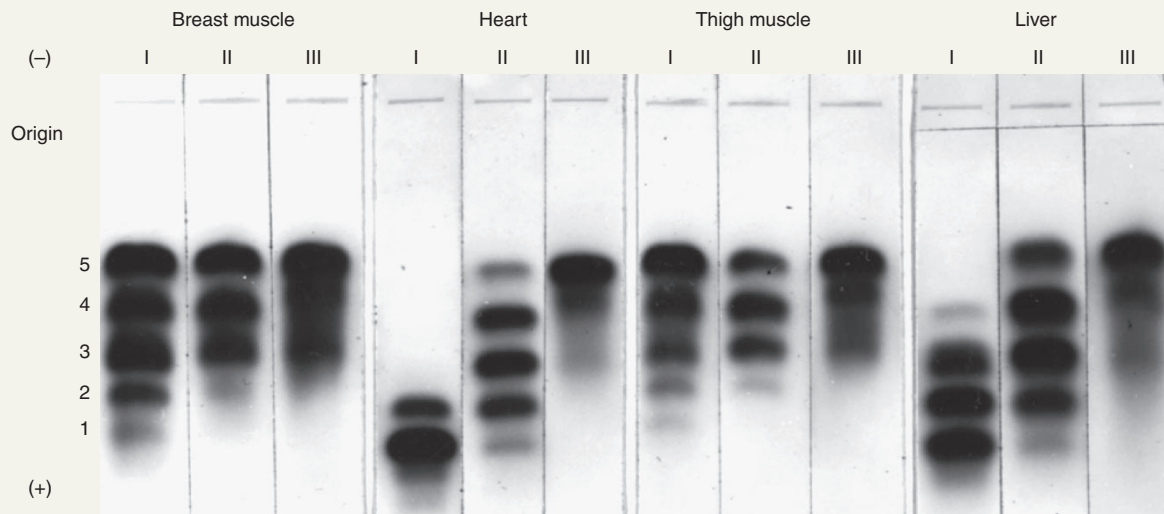


Figure 3 Lactate dehydrogenase isozyme patterns in pigeons. Note the five bands for some individual samples. Lanes *I*, *II*, and *III* under each tissue type indicate the range of individual variation. (W. H. Zinkham, et al., "A Variant of Lactate Dehydrogenase in Somatic Tissues of Pigeons" in *Journal of Experimental Zoology* 162, no. 1 (June):45–46, 1966. Reproduced by permission of the Wistar Institute.)

BOX 5.2 (CONTINUED)

Protein chemists have verified this tetramer model. In this way, electrophoresis has helped us determine the structure of several enzymes. (The term **isozymes** refers to multiple electrophoretic forms of an enzyme due to subunit interaction rather than allelic differences.)

Chemists have also discovered that the five forms differ in concentration in different tissues of the body (fig. 4). This has led to various hypotheses as to how the production of enzymes is controlled developmentally.

Electrophoresis is also valuable in clinical diagnosis. In various diseases,

cell destruction causes the release of proteins into the bloodstream. Thus, the lactate dehydrogenase pattern is found in the blood in certain disease states (fig. 5). This is why examination of the blood LDH is often a diagnostic test used to pick up early signs of heart and liver disease (among others).

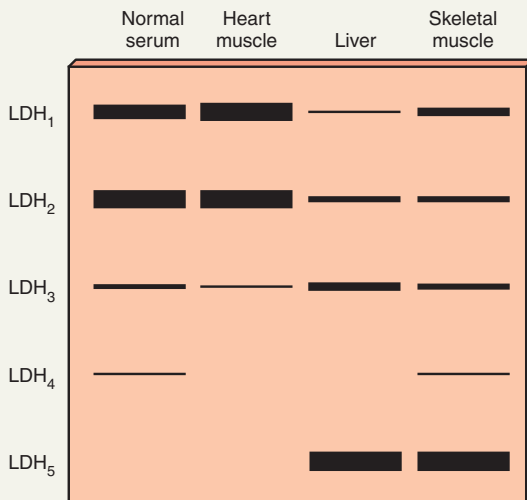


Figure 4 LDH patterns found in different tissues in human beings.

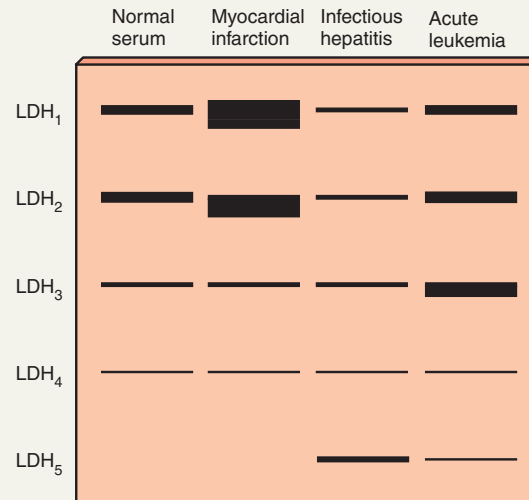


Figure 5 LDH patterns from normal human serum and from serum affected by various disease states.

The gene product of *XIST* is an RNA that does not seem to be translated into a protein. Rather, using localization techniques, geneticists have found this RNA is associated with Barr bodies, coating the inactive chromosome. Current research is aimed at determining the details of this interaction.

Dosage Compensation for *Drosophila*

Dosage compensation also occurs in fruit flies, and it appears that the gene activity of X chromosome loci is also about equal in males and females. The mechanism is different from that in mammals since no Barr bodies are found in fruit flies. Instead, the male's single X chromosome is hyperactive, approaching the level of transcriptional activity of both of the female's X chromosomes combined. Researchers have discovered a multisubunit protein complex called MSL (for male-specific lethal) that binds to

hundreds of sites on the single X chromosome in males. Presumably, the binding mediates the hyperactivity of the genes on the X chromosome. (We discuss control of transcription later in the book.) At least five genes contribute products to this protein complex: *mSl1*, *mSl2*, *mSl3*, *mle*, and *mof*. (*Mle* comes from *maleless*, and *mof* comes from *males absent on the first*.) Along with this protein complex are RNAs that also bind to the male X chromosome. These RNAs, also implicated in dosage compensation, are the products of the *rox1* and *rox2* genes (for *RNA on the X*). Together, the MSL protein complex and the RNAs comprise a **compensasome**.

Mutant alleles of the male-specific lethal (*mSl*) genes disrupt dosage compensation in males and are, as their names imply, lethal. However, they appear to have no effect in females. Expression of at least one of these genes, *mSl2*, is repressed by the protein product of the *Sxl* gene. Thus, sex determination and dosage compensation are



Figure 5.10 Tortoiseshell cat. A female heterozygous for the X-linked *yellow* and *black* alleles. (Courtesy of Donna Bass.)

ultimately under the control of the same master switch gene, *Sxl*. This should not be surprising since the ability of *Sxl* to count the number of X chromosomes in a cell makes it the most efficient initiator of both sexual development and dosage compensation.

SEX LINKAGE

In an XY chromosomal system of sex determination, the pattern of inheritance for loci on the heteromorphic sex chromosomes differs from the pattern for loci on the homomorphic autosomal chromosomes because alleles of the sex chromosome are inherited in association with the sex of the offspring. Alleles on a male's X chromosome go to his daughters but not to his sons, because the presence of his X chromosome normally determines that his offspring is a daughter. For example, the inheritance pattern of hemophilia (failure of blood to clot), the common form of which is caused by an allele located on the X chromosome, has been known since the end of the eighteenth century. It was known that mostly men had the disease, whereas women could pass on the disease without actually having it. (In fact, the general nature of the inheritance of this trait was known in biblical times. The Talmud—the

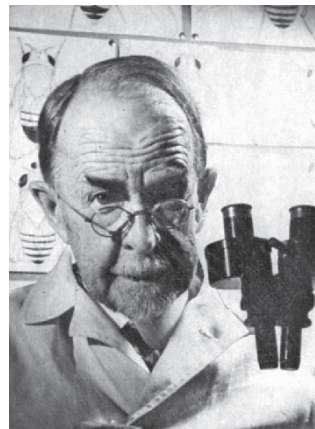
Jewish book of laws and traditions—specified exemptions to circumcision on the basis of hemophilia among relatives consistent with an understanding of who was at risk.)

Before we continue, we need to make a small distinction. Since both X and Y are sex chromosomes, three different patterns of inheritance are possible, all sex linked (for loci found only on the X chromosome, only on the Y chromosome, or on both). However, the term **sex-linked** usually refers to loci found only on the X chromosome; the term **Y-linked** is used to refer to loci found only on the Y chromosome, which control **holandric traits** (traits found only in males). Loci found on both the X and Y chromosomes are called *pseudoautosomal*. In human beings, at least four hundred loci are known to be on the X chromosome; only a few are known to be on the Y chromosome.

X Linkage in *Drosophila*

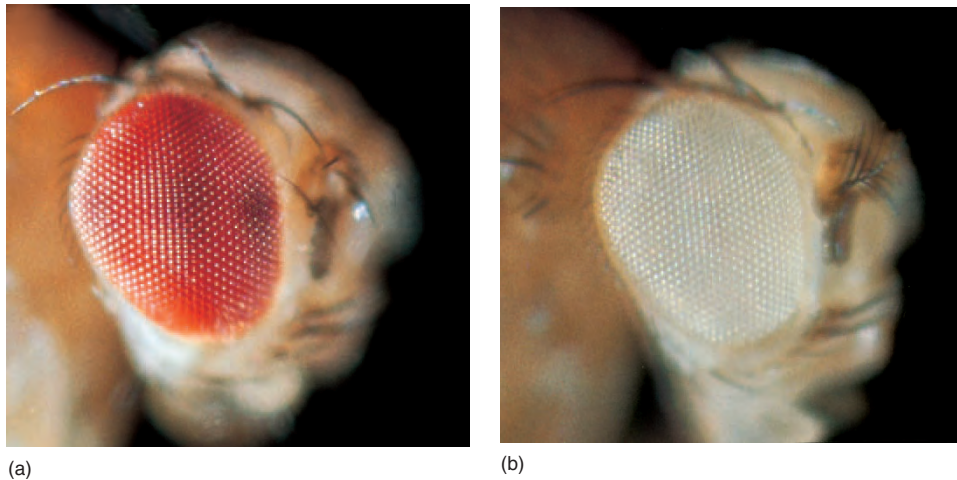
T. H. Morgan demonstrated the **X-linked** pattern of inheritance in *Drosophila* in 1910, when a white-eyed male appeared in a culture of wild-type (red-eyed) flies (fig. 5.11). This male was crossed with a wild-type female. All of the offspring were wild-type. However, when these F₁ individuals were crossed with each other, their offspring fell into two categories (fig. 5.12). All the females and half the males were wild-type, whereas the remaining half of the males were white-eyed. Ultimately, Morgan and others interpreted this to mean that the white-eye locus was on the X chromosome. We can redraw figure 5.12 to include the sex chromosomes of Morgan's flies (fig. 5.13). We denote the X chromosome with the white-eye allele as X^w. Similarly X⁺ is the X chromosome with the wild-type allele, and Y is the Y chromosome, which does not have this locus.

Another property of sex linkage appears in figure 5.13. Since females have two X chromosomes, they can have normal homozygous and heterozygous allelic combinations. But males, with only one copy of the X chromosome, can be neither homozygous nor heterozygous.



Thomas Hunt Morgan (1866–1945). (From *Genetics* 32 (1947): frontispiece. Courtesy of the Genetics Society of America.)

Figure 5.11 (a) Wild-type (red-eyed) and (b) white-eyed fruit flies. (Carolina Biological Supply Company.)



Instead, the term **hemizygous** describes the presence of X-linked genes (and other genes present in only one copy) in males. Since only one allele is present, a single copy of a recessive allele determines the phenotype in a phenomenon called **pseudodominance**. Thus, a male with one *w* allele is white-eyed, the allele acting in a dominant fashion. This is the same way one copy of a dominant autosomal allele would determine the phenotype of a normal diploid organism. Hence the term *pseudodominance*.

Nonreciprocity

The X-linked pattern has long been known as the **crisscross pattern of inheritance** because the father passes a trait to his daughters, who pass it to their sons. Figure 5.14 shows why this analysis is correct and the inheritance pattern is not reciprocal through a cross between a white-eyed female and a wild-type male. Here the F_1 males are white-eyed, the F_1 females are wild-type, and 50% of each sex in the F_2 generation are white-eyed. Such nonreciprocity and different ratios in the two sexes suggest sex linkage, which the crisscross pattern confirms.

Figure 5.15 shows the inheritance pattern of a sex-linked trait in chickens, in which the male is the homogametic sex (ZZ). The gene for barred plumage is Z linked, and barred plumage is dominant to nonbarred plumage. If we substitute white-eyed for nonbarred and male for female, we get the same pattern as in fruit flies (fig. 5.13)—in which, of course, females are homogametic.

The Y chromosome in fruit flies carries the pseudoautosomal *bobbed* locus (*bb*), the nucleolar organizer. In the homozygous recessive state, it causes bristles to shorten. Figures 5.16 and 5.17 show the results of reciprocal crosses involving *bobbed*. In both cases, one quarter of the F_2 individuals are *bobbed*. In one cross it is males, and in the other it is females.

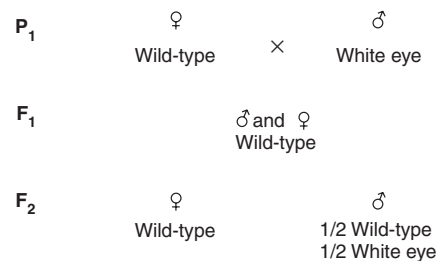


Figure 5.12 Pattern of inheritance of the white-eye trait in *Drosophila*.

Sex-Limited and Sex-Influenced Traits

Aside from X-linked, holandric, and pseudoautosomal inheritance, two inheritance patterns show nonreciprocity without necessarily being under the control of loci on the sex chromosomes. **Sex-limited traits** are traits expressed in only one sex, although the genes are present in both. In women, breast and ovary formation are sex-limited traits, as are facial hair distribution and sperm production in men. Nonhuman examples are plumage patterns in birds—in many species, the male is brightly colored—and horns found only in males of certain sheep species. Milk yield in mammals is expressed phenotypically only in females. **Sex-influenced**, or **sex-conditioned**, traits appear in both sexes but occur in one sex more than the other. Pattern, or premature, baldness in human beings is an example of a sex-influenced trait. In women, it is usually expressed as a thinning of hair rather than as balding. Apparently testosterone, the male hormone, is required for the full expression of the allele.

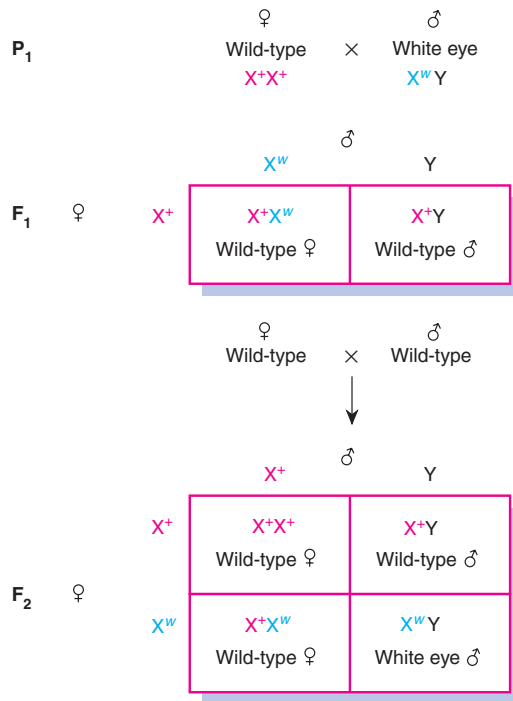


Figure 5.13 Crosses of figure 5.12 redrawn to include the sex chromosomes.

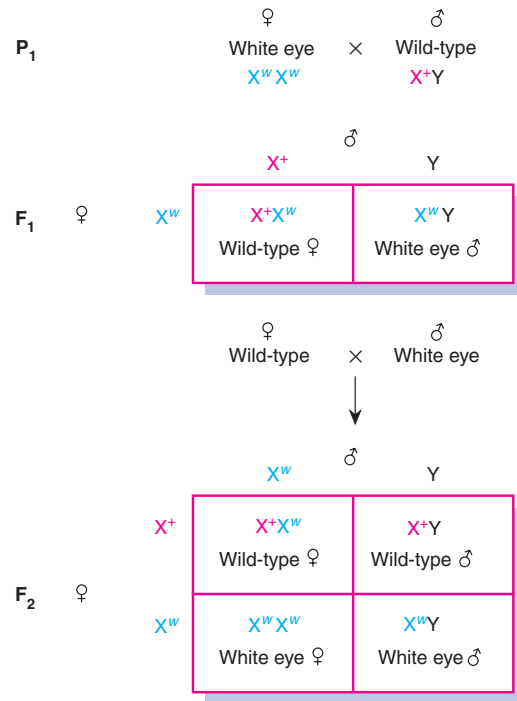


Figure 5.14 Reciprocal cross to that in figure 5.13.

PEDIGREE ANALYSIS

Inheritance patterns in many organisms are relatively easy to determine, because crucial crosses can test hypotheses about the genetic control of a particular trait. Many of these same organisms produce an abundance of offspring so that investigators can gather numbers large enough to compute ratios. Recall Mendel's work with garden peas; his 3:1 ratio in the F₂ generation led him to suggest the rule of segregation. If Mendel's sample sizes had been smaller, he might not have seen the ratio. Think of the difficulties Mendel would have faced had he decided to work with human beings instead of pea plants. Human geneticists face the same problems today. The occurrence of a trait in one of four children does not necessarily indicate a true 3:1 ratio.

To determine the inheritance pattern of many human traits, human geneticists often have little more to go on than a **pedigree** that many times does not include critical mating combinations. Frequently uncertainties and ambiguities plague pedigree analysis, a procedure whereby conclusions are often a product of the process of elimination. Other difficulties human geneticists encounter are the lack of **penetrance** and different degrees of **expressivity** in many traits. Both are aspects of the expression of a phenotype.

Penetrance and Expressivity

Penetrance refers to the appearance in the phenotype of genotypically determined traits. Unfortunately for geneticists, not all genotypes "penetrate" the phenotype. For example, a person could have the genotype that specifies vitamin-D-resistant rickets and yet not have rickets (a bone disease). This disease is caused by a sex-linked dominant allele and is distinguished from normal vitamin D deficiency by its failure to respond to low levels of vitamin D. It does, however, respond to very high levels of vitamin D and is thus treatable. In any case, in some family trees, affected children are born to unaffected parents. This would violate the rules of dominant inheritance because one of the parents must have had the allele yet did not express it. The fact that the parent actually had the allele is demonstrated by the occurrence of low levels of phosphorus in the blood, a pleiotropic effect of the same allele. The low-phosphorus aspect of the phenotype is always fully penetrant.

Thus, certain genotypes, often those for developmental traits, are not always fully penetrant. Most genotypes, however, are fully penetrant. For example, no known cases exist of individuals homozygous for albinism who do not actually lack pigment. Vitamin-D-resistant rickets illustrates another case in which a phenotype that is not genetically determined mimics a phenotype that is. This

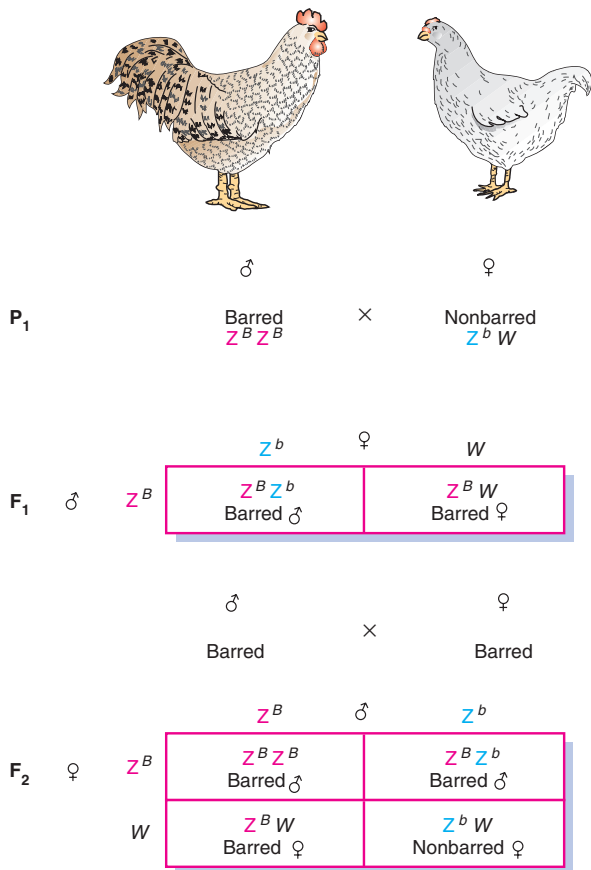


Figure 5.15 Inheritance pattern of barred plumage in chickens in which males are homogametic (ZZ) and females are heterogametic (ZW).

phenocopy is the result of dietary deficiency or environmental trauma. A dietary deficiency of vitamin D, for example, produces rickets that is virtually indistinguishable from genetically caused rickets.

Many developmental traits not only sometimes fail to penetrate, but also show a variable pattern of expression, from very mild to very extreme, when they do. For example, cleft palate is a trait that shows both variable penetrance and variable expressivity. Once the genotype penetrates, the severity of the impairment varies considerably, from a very mild external cleft to a very severe clefting of the hard and soft palates. Failure to penetrate and variable expressivity are not unique to human traits but are characteristic of developmental traits in many organisms.

Family Tree

One way to examine a pattern of inheritance is to draw a family tree. Figure 5.18 defines the symbols used in con-

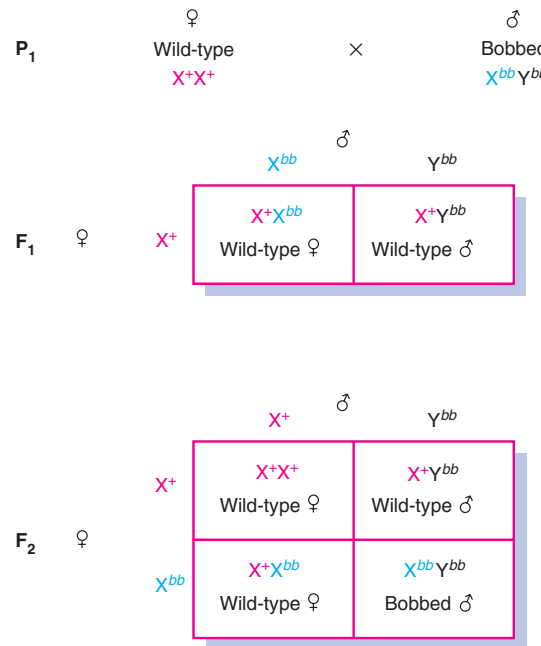


Figure 5.16 Inheritance pattern of the bobbed locus in *Drosophila*.

structing a family tree, or pedigree. The circles represent females, and the squares represent males. Symbols that are filled in represent individuals who have the trait under study; these individuals are said to be **affected**. The open symbols represent those who do not have the trait. Direct horizontal lines between two individuals (one male, one female) are called marriage lines. Children are attached to a marriage line by a vertical line. All the brothers and sisters (**siblings** or **sibs**) from the same parents are connected by a horizontal line above their symbols. Siblings are numbered below their symbols according to birth order (fig. 5.19), and generations are numbered on the right in Roman numerals. When the sex of a child is unknown, the symbol is diamond-shaped (e.g., the children of III-1 and III-2 in fig. 5.19). A number within a symbol represents the number of siblings not separately listed. Individuals IV-7 and IV-8 in figure 5.19 are fraternal (dizygotic or nonidentical) twins: they originate from the same point. Individuals III-3 and III-4 are identical (monozygotic) twins: they originate from the same short vertical line.

When other symbols occur in a pedigree, they are usually defined in the legend. Individual V-5 in figure 5.19 is called a **proband** or **propositus** (female, **proposita**). The arrow pointing to individual V-5 indicates that the pedigree was ascertained through this individual, usually by a physician or clinical investigator.

On the basis of the information in a pedigree, geneticists attempt to determine the mode of inheritance

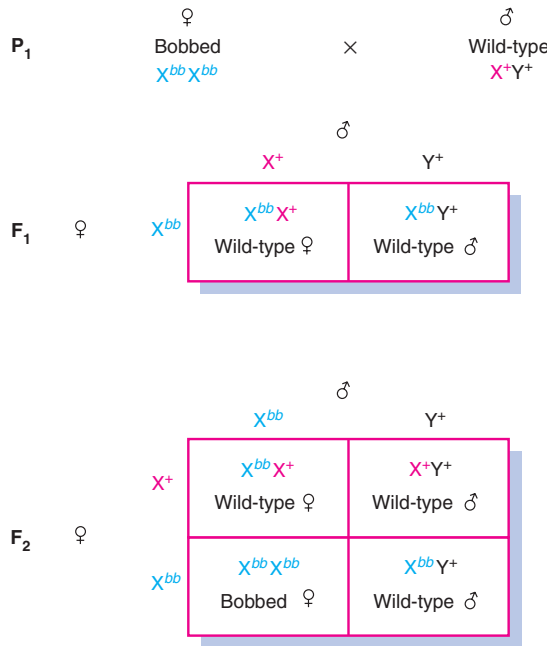


Figure 5.17 Reciprocal cross to that in figure 5.16.

of a trait. There are two types of questions the pedigree might be used to answer. First, are there patterns within the pedigree that are consistent with a particular mode of inheritance? Second, are there patterns within the pedigree that are inconsistent with a particular mode of inheritance? Often, it is not possible to determine the mode of inheritance of a particular trait with certainty. McKusick has reported that, as of 2001, the mode of inheritance of over nine thousand loci in human beings was known with some confidence, including autosomal dominant, autosomal recessive, and sex-linked genes.

Dominant Inheritance

If we look again at the pedigree in figure 5.19, several points emerge. First, polydactyly (fig. 5.20) occurs in every generation. Every affected child has an affected parent—no generations are skipped. This suggests dominant inheritance. Second, the trait occurs about equally in both sexes; there are seven affected males and six affected females in the pedigree. This indicates autosomal rather than sex-linked inheritance. Thus, so far, we would categorize polydactyly as an autosomal dominant trait. Note also that individual IV-11, a male, passed on the trait to two of his three sons. This would rule out sex linkage. (Remember that a male gives his X chromosome to all of his daughters but none of his

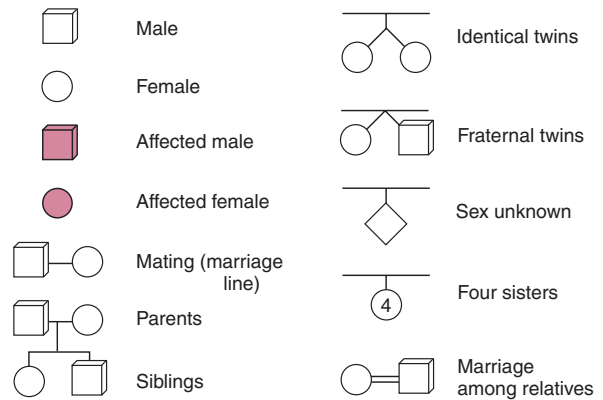


Figure 5.18 Symbols used in a pedigree.

sons. His sons receive his Y chromosome.) Consistency in many such pedigrees, has confirmed that an autosomal dominant gene causes polydactyly.

Polydactyly shows variable penetrance and expressivity. The most extreme manifestation of the trait is an extra digit on each hand (fig. 5.20) and one or two extra toes on each foot. However, some individuals have only extra toes, some have extra fingers, and some have an asymmetrical distribution of digits such as six toes on one foot and seven on the other.

Recessive Inheritance

Figure 5.21 is a pedigree with a different pattern of inheritance. Here affected individuals are not found in each generation. The affected daughters, identical triplets, come from unaffected parents. They represent, in fact, the first appearance of the trait in the pedigree. A telling point is that the parents of the triplets are first cousins; a mating between relatives is referred to as **consanguineous**. If the degree of relatedness is closer than law permits, the union is called **incestuous**. In all states, brother-sister and mother-son marriages are forbidden; and in all states except Georgia, father-daughter marriages are forbidden. Georgia did not intend to permit father-daughter marriages. However, the law was drafted using biblical terminology that inadvertently did not prohibit a man from marrying his daughter or his grandmother. Thirty states prohibit the marriage of first cousins.

Consanguineous matings often produce offspring that have rare recessive, and often deleterious, traits. The reason is that through common ancestry (e.g., when first cousins have a pair of grandparents in common), a rare allele can be passed on both sides of the pedigree and become homozygous in a child. The occurrence of a trait in a pedigree with common ancestry is often good evidence

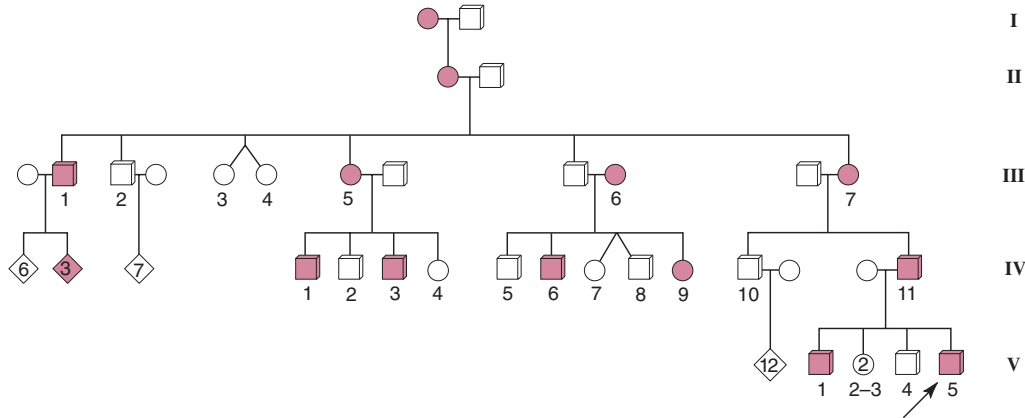


Figure 5.19 Part of a pedigree for polydactyly.

for an autosomal recessive mode of inheritance. Consanguinity by itself does not guarantee that a trait has an autosomal recessive mode of inheritance; all modes of inheritance appear in consanguineous pedigrees. Conversely, recessive inheritance is not confined to consanguineous pedigrees. Hundreds of recessive traits are known from pedigrees lacking consanguinity.

Sex-Linked Inheritance

Figure 5.22 is the pedigree of Queen Victoria of England. Through her children, hemophilia was passed on to many of the royal houses of Europe. Several interesting aspects of this pedigree help to confirm the method of inheritance. First, generations are skipped. Although Alexis (1904–18) was a hemophiliac, neither his parents nor his grandparents were. This pattern occurs in several other places in the pedigree and indicates a recessive mode of inheritance. From other pedigrees and from the biochemical nature of the defect, scientists have determined that hemophilia is a recessive trait.

Further inspection of the pedigree in figure 5.22 reveals that all the affected individuals are sons, strongly suggesting sex linkage. Since males are hemizygous for the X chromosome, more males than females should have the phenotype of a sex-linked recessive trait because males do not have a second X chromosome that might carry the normal allele. If this is correct, we can make several predictions. First, since all males get their X chromosomes from their mothers, affected males should be the offspring of carrier (heterozygous) females. A female is automatically a carrier if her father had the disease. She has a 50% chance of being a carrier if her brother, but not her father, has the disease. In that case, her mother was a carrier. The pedigree in figure 5.22 is consistent with these predictions.



Figure 5.20 Hands of a person with polydactyly. Manifestations range in severity from one extra finger or toe to one or more extra digits on each hand and foot. (© L.V. Bergman/The Bergman Collection.)

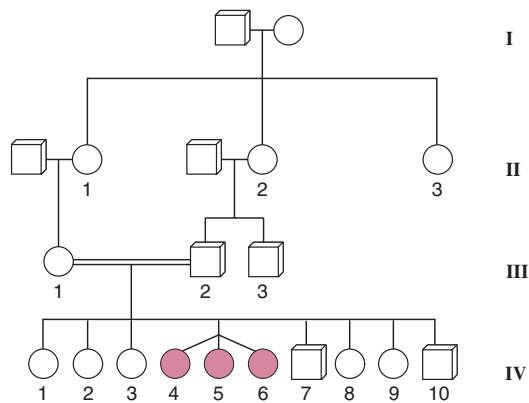
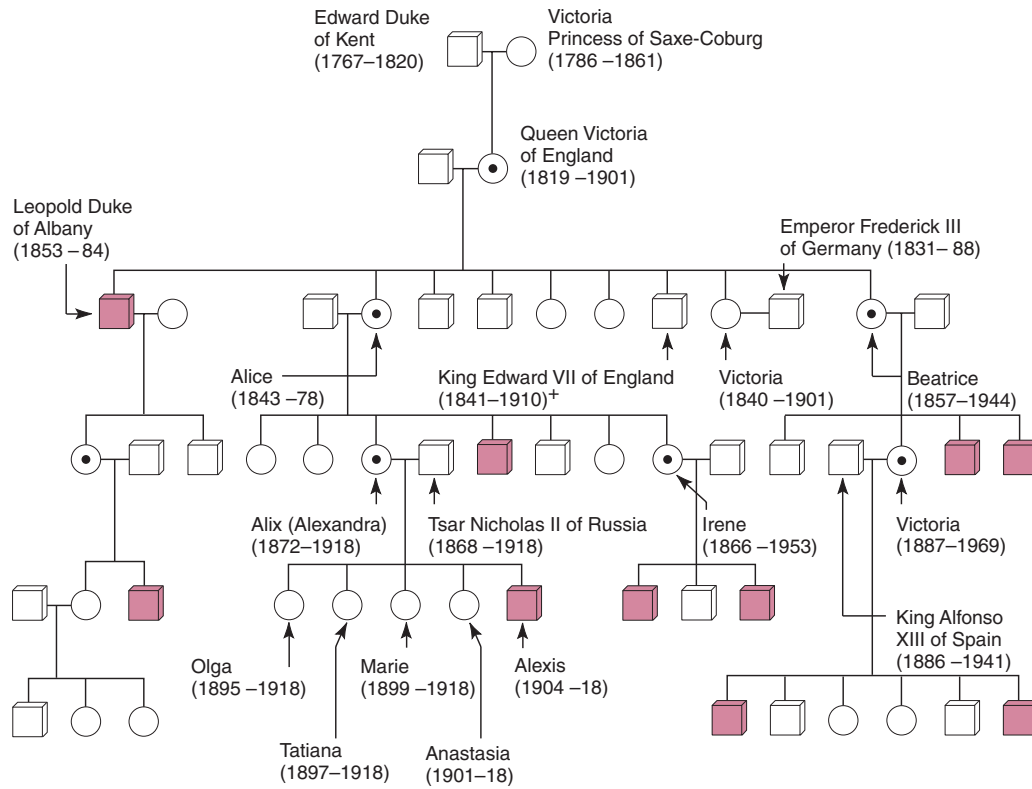


Figure 5.21 Part of a pedigree of hypotrichosis (early hair loss).



- Normal female
 - Normal, but known carrier (heterozygous) female
 - Normal male
 - Affected male
- + Descendants include present British royal family

Figure 5.22 Hemophilia in the pedigree of Queen Victoria of England. In the photograph of the Queen and some of her descendants, three carriers—Queen Victoria (*center*), Princess Irene of Prussia (*right*), and Princess Alix (Alexandra) of Hesse (*left*)—are indicated. (Photo © Mary Evans Picture Library/Photo Researchers, Inc.)

In no place in the pedigree is the trait passed from father to son. This would defy the route of an affected X chromosome. We can conclude from the pedigree that hemophilia is a sex-linked recessive trait. (Several different inherited forms of hemophilia are known, each deficient in one of the steps in the pathway that forms fibrinogen, the blood clot protein. Two of these forms, "classic" hemophilia A and hemophilia B, also called Christmas disease, are sex linked. Other hemophilias are autosomal.)

One other interesting point about this pedigree is that there is no evidence of the disease in Queen Victoria's ancestors, yet she was obviously a heterozygote, having one affected son and two daughters who were known carriers. Thus, though she was born to what appears to be a homozygous normal mother and a hemizygous normal father, one of Queen Victoria's X chromosomes had the hemophilia allele. This could have happened if a change (mutation) had occurred in one of the gametes that formed Queen Victoria. (We explore the mechanisms of mutation in chapter 12.)

Figure 5.23 is another pedigree that points to dominant inheritance because the trait skips no generations. The pedigree shows the distribution of low blood-phosphorus levels, the fully penetrant aspect of vitamin-D-resistant rickets, among the sexes. Affected males pass on the trait to their daughters but not their sons. This pattern follows that of the X chromosome: a male passes it on to all of his daughters but to none of his sons. Although this pedigree accords with a sex-linked dominant mode of inheritance, it does not rule out autosomal inheritance. The pedigree shown is a small part of one involving hundreds of people, all with phenotypes consistent with the hypothesis of sex-linked dominant inheritance.

In figure 5.23, there is the slight possibility that the trait is recessive. This could be true if the male in generation I and the mates of II-5 and II-7 were all heterozygotes. Since this is a rare trait, the possibility that all these conditions occurred is small. For example, if one person in fifty (0.02) is a heterozygote, then the probability of three heterozygotes mating within the same pedigree is $(0.02)^3$, or eight in one million. The rareness of this event further supports the hypothesis of dominant inheritance. The expected patterns for the various types of inheritance in pedigrees can be summarized in the following four categories:

Autosomal Recessive Inheritance

1. Trait often skips generations.
2. An almost equal number of affected males and females occur.
3. Traits are often found in pedigrees with consanguineous matings.
4. If both parents are affected, all children should be affected.

5. In most cases when unaffected people mate with affected individuals, all children are unaffected. When at least one child is affected (indicating that the unaffected parent is heterozygous), approximately half the children should be affected.
6. Most affected individuals have unaffected parents.

Autosomal Dominant Inheritance

1. Trait should not skip generations (unless trait lacks full penetrance).
2. When an affected person mates with an unaffected person, approximately 50% of their offspring should be affected (indicating also that the affected individual is heterozygous).
3. The trait should appear in almost equal numbers among the sexes.

Sex-Linked Recessive Inheritance

1. Most affected individuals are male.
2. Affected males result from mothers who are affected or who are known to be carriers (heterozygotes) because they have affected brothers, fathers, or maternal uncles.
3. Affected females come from affected fathers and affected or carrier mothers.
4. The sons of affected females should be affected.
5. Approximately half the sons of carrier females should be affected.

Sex-Linked Dominant Inheritance

1. The trait does not skip generations.
2. Affected males must come from affected mothers.
3. Approximately half the children of an affected heterozygous female are affected.
4. Affected females come from affected mothers or fathers.
5. All the daughters, but none of the sons, of an affected father are affected.

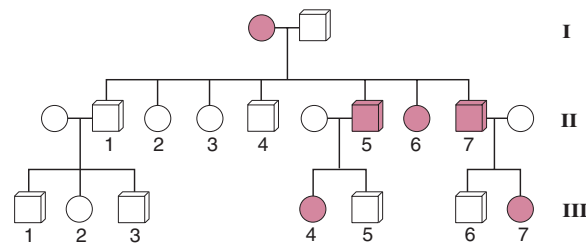


Figure 5.23 Part of a pedigree of vitamin-D-resistant rickets. Affected individuals have low blood-phosphorus levels. Although the sample is too small for certainty, dominance is indicated because every generation was affected, and sex linkage is suggested by the distribution of affected individuals.

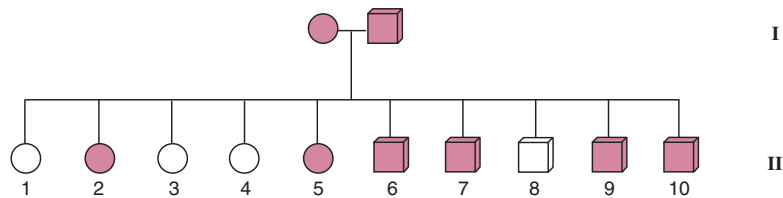


Figure 2 A pedigree for alcoholism.

Nor can the mode of inheritance be by a sex-linked dominant gene because an affected male would have only affected daughters, since his daughters get copies of his single X chromosome. We are thus left with autosomal dominance as the mode of inheritance. If that is the case, then both parents must be heterozygotes; otherwise, all the children would be affected. If both parents are heterozygotes, we expect a 3:1 ratio of affected to unaffected offspring (a cross of $Aa \times Aa$ produces offspring of $A:aa$ in a 3:1 ratio); here, the ratio is 6:4. If we did a chi-square test, the expected numbers would be 7.5:2.5 ($3/4$ and $1/4$, respectively, of 10). Although the expected value of 2.5 makes it inappropriate to do a chi-square test (the expected value is too small), we can see that the observed and expected numbers are very close. Thus, from the pedigree we would conclude that an autosomal dominant allele controls chronic alcoholism. (Although the analysis is consistent, we actually cannot draw that conclusion about alcoholism because other pedigrees are not consistent with 100% penetrance, a one-gene model, or the lack of environmental influences. In fact, scientists are currently debating whether alcoholism is inherited at all. These types of problems related to complex human traits are discussed in chapter 18.)

PROBLEM 3: A female fly with orange eyes is crossed with a male fly with short wings. The F_1 females have wild-type (red) eyes and long wings; the F_1 males have orange eyes and long wings. The F_1 flies are crossed to yield

47 long wings, red eyes
45 long wings, orange eyes
17 short wings, red eyes
14 short wings, orange eyes

with no differences between the sexes. What is the genetic basis of each trait?

Answer: In the F_1 flies, we see a difference in eye color between the sexes, indicating some type of sex linkage. Since the females are wild-type, wild-type is probably dominant to orange. We can thus diagram the cross for eye color as

(female) X^oX^o orange	↓	X^+Y (male) red	P ₁
X^+X^o red	↓	X^oY orange	F ₁
X^+X^o X^oX^o red orange	↓	X^+Y X^oY red orange	F ₂

We would thus expect to see equal numbers of red-eyed and orange-eyed males and females, which is what we observe. Now look at long versus short wings. If we disregard eye color, wing length seems to be under autosomal control with short wings being recessive. Thus, the parents are homozygotes (ss and s^+s^+), the F_1 offspring are heterozygotes (s^+s), and the F_2 progeny have a phenotypic ratio of 3:1, wild-type (long) to short wings.

EXERCISES AND PROBLEMS*

SEX DETERMINATION

1. What is the difference between an X and a Z chromosome?
2. Transformer (*tra*) is an autosomal recessive gene that converts chromosomal females into sterile males. A female *Drosophila* heterozygous for the transformer allele (*tra*) is mated with a normal male homozygous for transformer. What is the sex ratio of their offspring? What is the sex ratio of their offspring's offspring?

3. The autosomal recessive doublesex (*dsx*) gene converts males and females into developmental intersexes. Two fruit flies, both heterozygous for the *doublesex* (*dsx*) allele, are mated. What are the sexes of their offspring?
4. What is a sex switch? What genes serve as sex switches in human beings and *Drosophila*?

*Answers to selected exercises and problems are on page A-4.

DOSAGE COMPENSATION

5. The diagram of an electrophoretic gel in figure 3 shows activity for a particular enzyme. Lane 1 is a sample from a “fast” homozygote. Lane 2 is a sample from a “slow” homozygote. In lane 3, the blood from the first two was mixed. Lane 4 comes from one of the children of the two homozygotes.

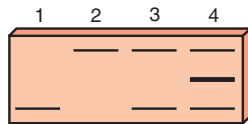


Figure 3 The activity of a particular enzyme as revealed on an electrophoretic gel.

Can you guess the structure of the enzyme? If this were an X-linked trait, what pattern would you expect from a heterozygous female's

- whole blood?
 - individual cells?
6. How many different zones of activity (bands) would you see on a gel stained for lactate dehydrogenase (LDH) activity from a person homozygous for the A protein gene but heterozygous for the B protein gene? Are the bands due to the activity of allozymes or isozymes?
7. How many Barr bodies would you see in the nuclei of persons with the following sex chromosomes?
- X0
 - XX
 - XY
 - XXY
 - XXX
 - XXXXX
 - XX/XY mosaic
- What would the sex of each of these persons be? If these were the sex chromosomes of individual *Drosophila* that were diploid for all other chromosomes, what would their sexes be?

8. Calico cats have large patches of colored fur. What does this indicate about the age of onset of Lyonization (is it early or late)? Tortoiseshell cats have very small color patches. Explain the difference between the two phenotypes.

SEX LINKAGE

9. In *Drosophila*, the lozenge phenotype, caused by a sex-linked recessive allele (*lz*), is narrow eyes. Diagram to the F₂ generation a cross of a lozenge male and a homozygous normal female. Diagram the reciprocal cross.
10. Sex linkage was originally detected in 1906 in moths with a ZW sex-determining mechanism. In the current moth, a pale color (*p*) is recessive to the wild-type and located on the Z chromosome. Diagram reciprocal crosses to the F₂ generation in these moths.
11. What family history of hemophilia would indicate to you that a newborn male baby should be exempted from circumcision?
12. What is the difference between *pseudodominance* and *phenocopy*?
13. In *Drosophila*, cut wings are controlled by a recessive sex-linked allele (*ct*), and fuzzy body is controlled by a recessive autosomal allele (*fy*). When a fuzzy female is mated with a cut male, all the members of the F₁ generation are wild-type. What are the proportions of F₂ phenotypes, by sex?
14. Consider the following crosses in canaries:
- | Parents | Progeny |
|--|--|
| a. pink-eyed female
× pink-eyed male | all pink-eyed |
| b. pink-eyed female
× black-eyed male | all black-eyed |
| c. black-eyed female
× pink-eyed male | all females pink-eyed,
all males black-eyed |
- Explain these results by determining which allele is dominant and how eye color is inherited.
15. Consider the following crosses involving yellow and gray true-breeding *Drosophila*:
- | Cross | F ₁ | F ₂ |
|---------------------------|---------------------------------------|---|
| gray female × yellow male | all males gray,
all females gray | 97 gray females,
42 yellow males,
48 gray males |
| yellow female × gray male | all females gray,
all males yellow | ? |
- Is color controlled by an autosomal or an X-linked gene?
 - Which allele, gray or yellow, is dominant?
 - Assume 100 F₂ offspring are produced in the second cross. What kinds and what numbers of progeny do you expect? List males and females separately.
16. A man with brown teeth mates with a woman with normal white teeth. They have four daughters, all with brown teeth, and three sons, all with white teeth. The sons all mate with women with white teeth, and all their children have white teeth. One of the daughters (A) mates with a man with white teeth (B), and they have two brown-toothed daughters, one white-toothed daughter, one brown-toothed son, and one white-toothed son.
- Explain these observations.
 - Based on your answer to *a*, what is the chance that the next child of the A-B couple will have brown teeth?
17. In human beings, red-green color blindness is inherited as an X-linked recessive trait. A woman with normal vision whose father was color-blind marries a man with normal vision whose father was also color-

blind. This couple has a color-blind daughter with a normal complement of chromosomes. Is infidelity suspected? Explain.

18. A white-eyed male fly is mated with a pink-eyed female. All the F₁ offspring have wild-type red eyes. F₁ individuals are mated among themselves to yield:

Females		Males	
red-eyed	450	red-eyed	231
pink-eyed	155	white-eyed	301
		pink-eyed	70

Provide a genetic explanation for the results.

19. In *Drosophila*, white eye is an X-linked recessive trait, and ebony body is an autosomal recessive trait. A homozygous white-eyed female is crossed with a homozygous ebony male.
- What phenotypic ratio do you expect in the F₁ generation?
 - What phenotypic ratio do you expect in the F₂ generation?
 - Suppose the initial cross was reversed: ebony female × white-eyed male. What phenotypic ratio would you expect in the F₂ generation?

20. In *Drosophila*, abnormal eyes can result from mutations in many different genes. A true-breeding wild-type male is mated with three different females, each with abnormal eyes. The results of these crosses are as follows:

	Females	Males
male × abnormal-1	all normal	all normal
male × abnormal-2	1/2 normal, 1/2 abnormal	1/2 normal, 1/2 abnormal
male × abnormal-3	all abnormal	all abnormal

Explain the results by determining the mode of inheritance for each abnormal trait.

21. A black and orange female cat is crossed with a black male, and the progeny are as follows:
 females: two black, three orange and black
 males: two black, two orange

Explain the results.

22. Based on the following *Drosophila* crosses, explain the genetic basis for each trait and determine the genotypes of all individuals:

white-eyed, dark-bodied female × red-eyed, tan-bodied male

F₁: females are all red-eyed, tan-bodied; males are all white-eyed, tan-bodied

F₂: 27 red-eyed, tan-bodied
 24 white-eyed, tan-bodied
 9 red-eyed, dark-bodied
 7 white-eyed, dark-bodied

(No differences between males and females in the F₂ generation.)

PEDIGREE ANALYSIS

23. What is the difference between penetrance and expressivity?
24. What are the possible modes of inheritance in pedigrees a–c in figure 4? What modes of inheritance are not possible for a given pedigree?

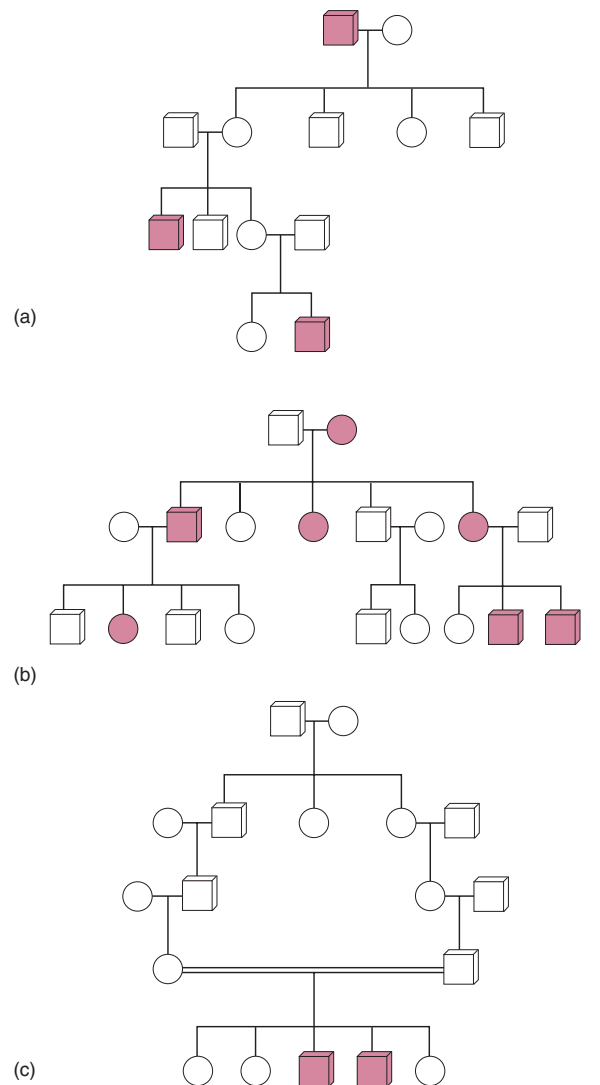


Figure 4 Three pedigrees showing different modes of inheritance.

25. In pedigrees *a–d* in figure 5, which show the inheritance of rare human traits, including twin production, determine which modes of inheritance are most probable, possible, or impossible.
26. Hairy ears, a human trait expressed as excessive hair on the rims of ears in men, shows reduced penetrance (less than 100% penetrant). Mechanisms proposed include Y linkage, autosomal dominance, and

autosomal recessiveness. Construct a pedigree consistent with each of these mechanisms.

27. Construct pedigrees for traits that *could not be*

- a. autosomal recessive.
- b. autosomal dominant.
- c. sex-linked recessive.
- d. sex-linked dominant.

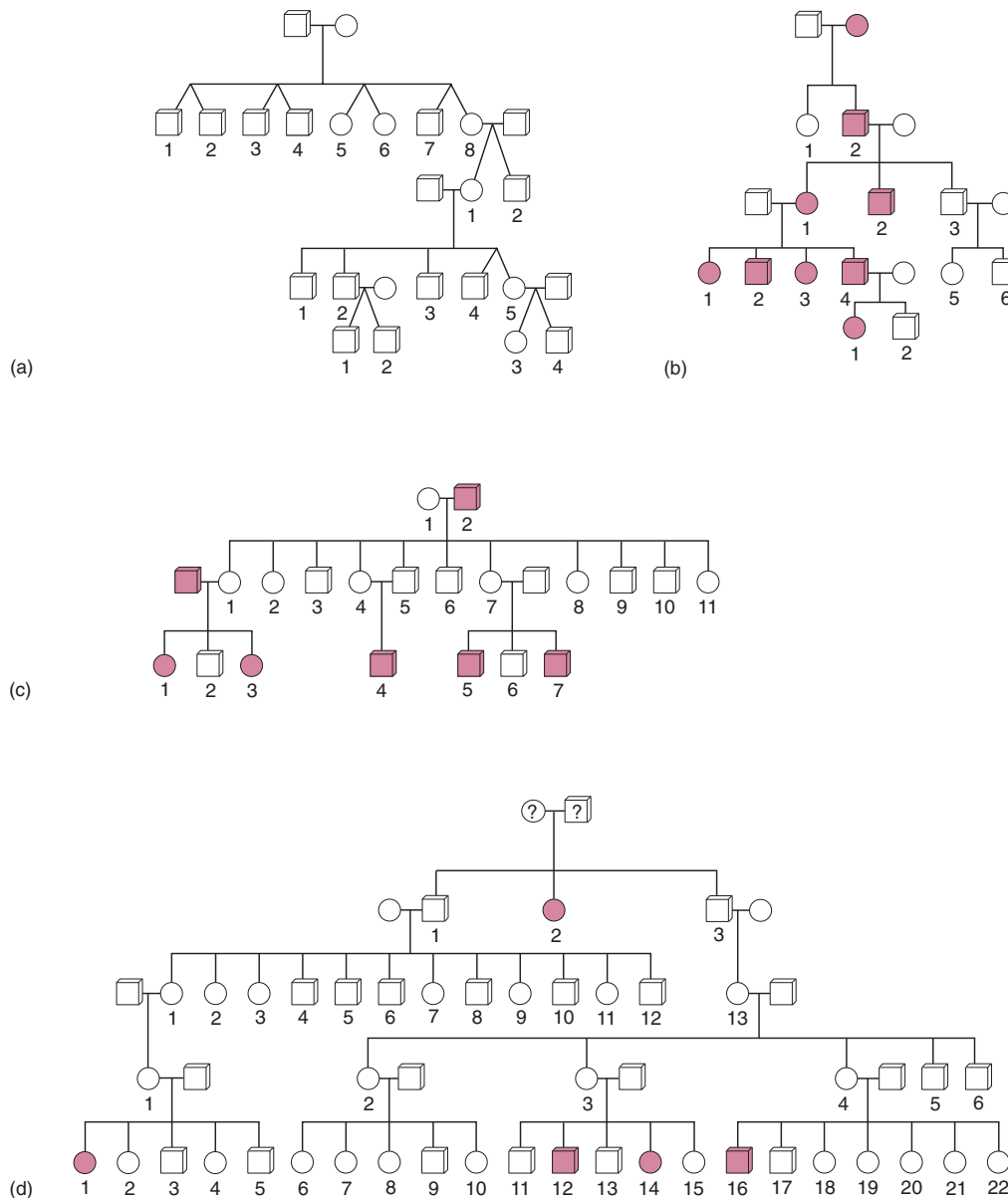


Figure 5 Pedigrees of rare human traits, including twin production (a).

28. Determine the possible modes of inheritance for each trait in pedigrees a–c in figure 6.

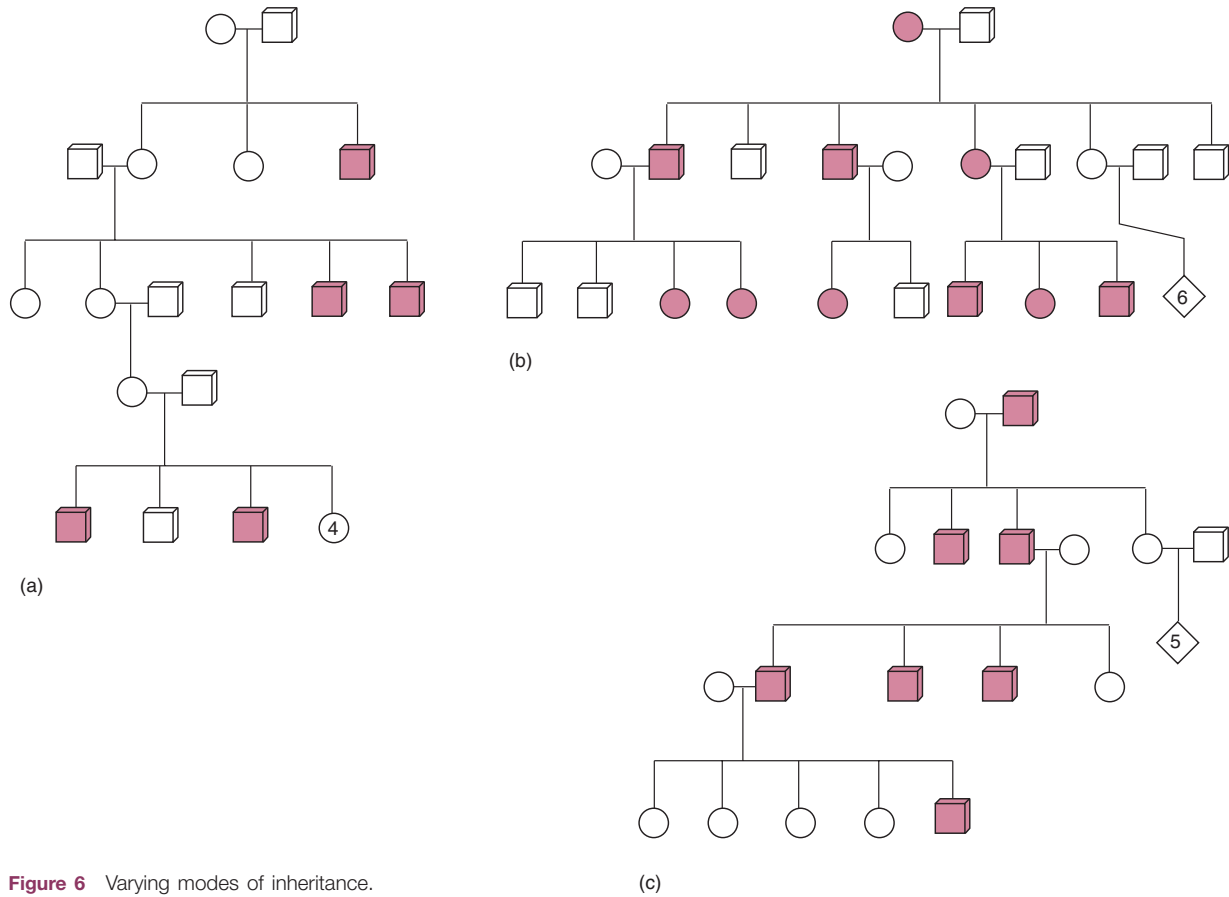


Figure 6 Varying modes of inheritance.

CRITICAL THINKING QUESTIONS

1. What effects do null alleles, alleles that produce no protein product, have in electrophoretic systems? How could you tell if a null allele were present?
2. In 1918, the Bolsheviks killed Tsar Nicholas II of Russia and his family (fig. 5.22). However, the remains of one

daughter, Princess Anastasia, were never recovered. At one point, a woman appeared who claimed to be Anastasia. How could you validate her claim genetically?