

# PRINCIPLES OF NEURAL SCIENCE

Fourth Edition

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## Genes and Behavior

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Mutations in the Gene Encoding Leptin Affect Feeding Behavior

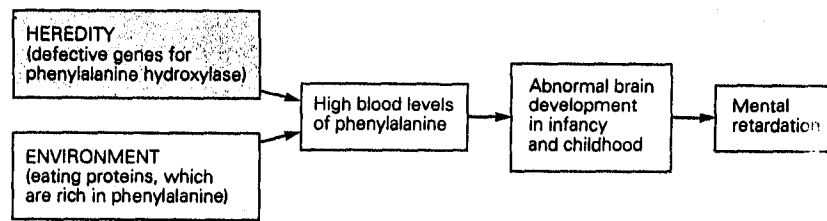
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**A**LL BEHAVIOR IS SHAPED BY the interplay of genes and the environment. Even the most stereotypic behaviors of simple animals can be influenced by the environment, while highly evolved behaviors in humans, such as language, are constrained by hereditary factors. In this chapter we review what is known about the role of genes in organizing behavior. Later in the book we discuss the role of environmental factors.

A striking illustration of how genes and environment interact is evident in phenylketonuria. This disease results in a severe impairment of cognitive function and affects 1 child in 15,000. Children who express this disease have two abnormal copies of the gene that codes for phenylalanine hydroxylase, the enzyme that converts the amino acid phenylalanine, a component of dietary proteins, to another amino acid, tyrosine. Many more children carry only one abnormal copy of the gene

Figure 3-1 Heredity and environment are both necessary for the expression of phenylketonuria. (From Barondes 1995.)



tors in the diet are clearly necessary for the expression of this form of mental retardation. A mere change in diet can rescue the genetic defect and the mental functioning.

In considering genetic factors that control behavior we need first to identify the components of behavior that are heritable. Clearly, behavior itself is not inherited; what is inherited is DNA, which encodes proteins. The genes expressed in neurons encode proteins that are important for development, maintenance, and regulation of the neural circuits that underlie all aspects of behavior. In turn, neural circuits are composed of many nerve cells, each of which expresses a special constellation of genes that direct the production of specific proteins. For the development and function of a single neural circuit, a wide variety of structural and regulatory proteins are required. In simple animals a single gene may control a behavioral trait by encoding a protein that affects the function of individual nerve cells in a specific neural circuit. In more complex animals the circuitry is also more complex and behavioral traits are generally shaped by the actions of many genes. Subtle differences in behavior can be achieved not only by the presence or absence of a given gene product or a set of products, but also by the *degree* to which different gene products are expressed, or by the specific contribution of gene products.

The interplay of the genes, proteins, and neural circuits underlying behavior has been studied in various organisms ranging in complexity from worms and flies to mice and humans. Molecular genetics provides the techniques to identify the genes involved in a particular behavior and to determine how the proteins they encode control behavior. In worms, flies, and even in vertebrate organisms such as mice and zebrafish, it is possible to examine directly how genes influence behavior because single-gene mutants of these organisms can be bred and isolated.

In this chapter we illustrate how the genetic dissection of behavior in simple animals can provide insight into the mechanisms that regulate human behavioral traits. We then discuss a few important examples of the effects of single-gene defects on human behavior. Finally,

we consider complex behavioral traits that typically are determined by the actions of many genes.

### Genetic Information Is Stored in Chromosomes

Genes contribute to the neural circuitry of behavior in two fundamental ways. First, through their ability to replicate reliably, each gene provides precise copies of itself to all cells in an organism as well as succeeding generations of organisms. Second, each gene that is expressed in a cell directs the manufacture of specific proteins that determine the structure, function, and other biological characteristics of the cell.

With rare exceptions, each cell in the human body contains precisely the same complement of genes, thought to be about 80,000. The reason cells differ from one another—why one cell becomes a liver cell and another a brain cell—is that a distinct set of genes is expressed (as messenger RNA) in each cell type. Which genes and proteins become activated in a particular cell depends on interactions between the molecules within the cell, between neighboring cells, and between the cell and the organism's external environment (see Chapter 52). More of the total genetic information encoded in DNA—perhaps 30,000 of the 80,000 genes—is expressed in brain cells than in any other tissue of the body. Genes vary in size from 1 to 200,000 kilobases; the average size is about 10 kilobases. The DNA of each gene that encodes a protein is made up of segments, called *exons*, which encode parts of the protein and these coding segments are interrupted by noncoding segments called *introns*.

DNA is not distributed randomly within the nucleus but arranged in an orderly way on structures called chromosomes. The number of chromosomes varies among different organisms. In addition, different types of organisms contain either one or two copies of each chromosome. With some exceptions, unicellular organisms are *haploid*; they have only a single copy of each chromosome. By contrast, most complex multicellular organisms (worms, fruit flies, mice, and hu-

mans) are *diploid*; in all their somatic cells they carry two homologous copies of each chromosome and each gene, one from the mother and the other from the father.

The number of chromosomes in the *germ*, or sex, cells (sperm and egg) is half that found in somatic cells. During the nuclear division that accompanies somatic cell division (the process of mitosis) the chromosomes are partitioned equally—each daughter cell receives one copy of each chromosome in the parent cell. However, during the two successive nuclear divisions that accompany division of the germ cells (meiosis), the number of chromosomes is reduced by half. Fertilization of the egg by the sperm restores the diploid number found in somatic cells, with homologous chromosomes contributed by each parent.

The 80,000 genes in the human genome are arranged in a precise order along the chromosomes. As a result, each gene is uniquely identifiable by its location at a characteristic position (*locus*) on a specific chromosome. The two copies of a gene at corresponding loci on a pair of homologous chromosomes commonly harbor sequence variations, or polymorphisms, at multiple sites throughout the gene. At any given site, the alternative gene versions are referred to as *alleles*. Alleles may be identical or, more commonly, differ to some degree because of polymorphisms or mutations, as discussed below.

If two alleles are identical, the organism is said to be *homozygous* at that locus. If the alleles vary in form (in their nucleotide sequence), the organism is said to be *heterozygous* at that locus. The recent DNA sequencing of a small number of human genes reveals large variance in the degree of intergenic polymorphism. In general, however, the rate of polymorphic variation between any two individuals is estimated to be 1 per 1000 base pairs in noncoding DNA and 1 per 2000 base pairs in coding DNA. Thus a 10 kilobase gene would harbor, on average, about 10 polymorphisms, including 1 or 2 in the coding sequence DNA. At each of these polymorphic sites, an individual will carry at most two different forms of the same allele, whereas the same allele may exist in many forms within a population. A difference within a population is called *allelic polymorphism*, or more generally, *genetic polymorphism*. Prominent examples of allelic polymorphism are the alleles of the genes responsible for hair and eye color.

Humans have 46 chromosomes: 22 pairs of autosomes and two sex chromosomes (two X chromosomes in females, one X and one Y chromosome in males). The parents contribute the sex chromosomes to their offspring differently from the manner they supply the autosomes. A spermatozoon carries either an X (female-determining) or a Y (male-determining) chromosome, whereas an ovum carries only an X chromosome. As a consequence, males inherit their single X chromosome from their mothers.

The 22 autosome pairs and the X and Y sex chromosomes vary in size and cytological banding pattern (Figure 3-2). Chromosome 1 is the largest autosome; it contains 8% of the human genome, or about 6400 genes. Chromosome 22 is the smallest, containing 1% or about 800 genes. Chromosomes also vary in the nucleotide sequence of their DNA, but paired autosomes are usually morphologically (cytogenetically) indistinguishable.

### Gregor Mendel's Work Led to the Delineation of the Relationship Between Genotype and Phenotype

The existence of alternative allelic forms of genes were discovered in 1866 by Gregor Mendel, who demonstrated the difference between dominant and recessive alleles using garden peas as an experimental system. Mendel started out with self-breeding experiments on peas. These led to the creation of inbred strains of peas that bred true for given characteristics of the pea such as color or the shape of the pod. He then crossed these inbred strains with each other and observed how the various traits were manifested in the progeny of the pea plant. These crosses allowed Mendel to appreciate that the variability in heredity among the progeny lay in differences in discrete factors that are passed unchanged from one plant generation to another, factors we now call genes. Moreover, Mendel found that each pea had two sets of factors, one from the male parent and the other from the female.

Mendel carried out his studies before it was known how chromosomes behave during cell division. Forty years later it became clear that the segregation pattern of genes noted by Mendel paralleled, almost exactly, the behavior of chromosomes during meiotic cell division, the division that produces the male and female germ cells. These findings were used by Thomas Hunt Morgan to formulate the *chromosomal theory of heredity*, according to which each chromosome has a linear array of unique genes running from one end to the other, each gene having a definite location on a particular chromosome.

While studying Mendel's results, Wilhelm Johannsen later distinguished between the *genotype* of an organism (its genetic makeup) and the *phenotype* of an organism (its appearance). In the broad sense *genotype* refers to the entire set of alleles forming the genome of an individual; in the narrow sense it refers to the specific alleles of one gene. *Phenotype* denotes the functional expression or consequences of a gene or set of genes. The phenotype of an individual may change throughout life, whereas the genotype remains constant except for sporadic mutations.

Most mutations are simply allelic polymorphisms that are silent; that is, they do not have any effect on the phenotype. Some are not silent but are expressed in ways that nevertheless appear neutral and therefore be-

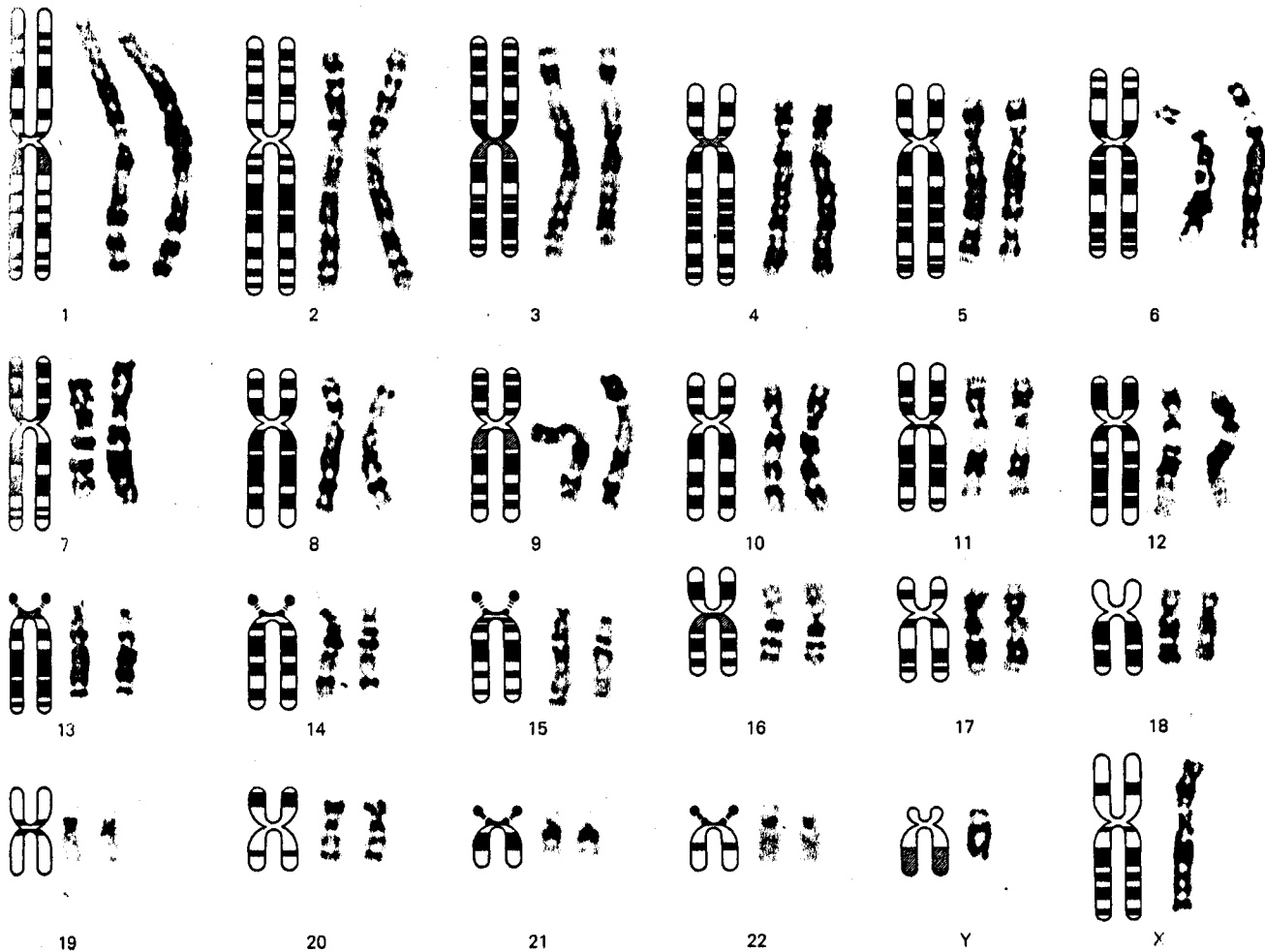


Figure 3-2 Map of normal human chromosomes at metaphase illustrating the distinctive morphology of each chromosome. (Adapted from Watson et al. 1983.)

nign (Box 3-1). Benign mutations are allelic polymorphisms that produce differences in body type, such as eye color or hair color, as well as differences in personality characteristics. The consequence of a mutation is often shaped by the environment. A mutation that favored a hunter-gatherer's survival during periodic food shortages might lead to pathological obesity in a modern-day environment. Many mutations that do not have benign consequences, such as those leading to excessive tallness, dwarfism, or color blindness, do not necessarily impair everyday functions. Some mutations may have significant consequences that are limited to the cell-biological level, without any functional effects. An example would be a mutation that results in the failure of a single type of cell to develop in an animal that can compensate for the loss of that cell type. Only rarely do mutations lead to significant changes in development, cell function, or overt behavior. Some mutations

are truly pathogenic, however, and these lead to human disease.

If a mutant phenotype results from one mutant allele in combination with one wild-type (normal) allele, the mutation or phenotypic trait is said to be *dominant*. Dominant mutations usually lead to the production of an abnormal protein by the mutant allele or to the expression of the wild-type gene product at an inappropriate time or place. Because they give rise to a new, perhaps toxic, variant of the protein or a new pattern of expression in the body, dominant mutations are often referred to as *gain of function* mutations. Some dominant mutations produce an inactive protein product that can nevertheless interfere with the function of the wild-type protein, thus leading to a complete loss of function of the gene. Such mutations are termed *dominant negative mutations*.

If a mutant phenotype is expressed only when both alleles of a gene are mutated (that is, only individuals

### Box 3-1 The Origins of Genetic Diversity

Although DNA replication generally is carried out with high fidelity, spontaneous errors called *mutations* do occur. Mutations may result from damage to the purine and pyrimidine bases, mistakes during the DNA replication process, and recombinations that occur between two nonhomologous chromosomes as a result of errors in crossing over during meiosis. It is these mutations that give rise to genetic polymorphisms.

The rate of spontaneously occurring mutations is low. However, the frequency of mutations greatly increases when the organism is exposed to chemical mutagens or ionizing radiation. Chemical mutagens tend to induce *point mutations* involving changes in a single DNA base pair or the deletion of a few base pairs. By contrast, ionizing radiation can induce large insertions, deletions, or translocations. Both spontaneous and induced mutations can lead to changes in the structure of the protein encoded by the gene (as in a dominant mutation) or to a partial decrease or absence of gene function or expression (as in recessive mutations).

Changes in a single base pair involve one of three types of point mutations: (1) a *missense mutation*, where the point muta-

tion results in one amino acid in a protein being substituted for another; (2) a *nonsense mutation*, where a stop codon (triplet) is substituted for a codon within the coding region, thus resulting in a shortened (truncated) protein product; or (3) a *frameshift mutation*, in which small insertions or deletions change the reading frame, leading to the production of a truncated or abnormal protein.

Large-scale mutations involve changes in chromosome structure that can affect the function of many contiguous genes. Such mutations include rearrangement of genes without the addition or deletion of material (*inversion*), *duplication* of genes in a chromosome, or the exchange (*crossing over*) between segments of DNA. Sometimes large deletions of multiple genes occur. While these mutations are usually fatal if present in both copies of a gene (homozygous lethals), they can result in phenotypes in the heterozygous state (such as the mental retardation associated with the Wilms tumor deletion complex). Chromosomal translocation can also cause fusion between different (nonhomologous) chromosomes.

homozygous for the mutant allele will exhibit the phenotype), the mutation or phenotypic trait is said to be *recessive*. Recessive mutations usually result from the loss or reduction in amount of a functional protein. As a result, recessive mutations are often *loss of function* mutations. The reason both alleles need to be defective in a recessive mutation in order for a phenotype to become evident is that a 50% reduction of most proteins (such as most enzymes) usually does not cause serious (or even detectable) problems in cell function.

#### The Genotype Is a Significant Determinant of Human Behavior

Independent of Mendel's work, Francis Galton began to apply genetics to human behavior in 1869. In his book *Hereditary Genius*, Galton proposed that relatives of individuals with extremely high mental ability were more likely to be endowed with similar abilities than would be predicted by chance: the closer the family relationship, the higher the incidence of such gifted individuals.

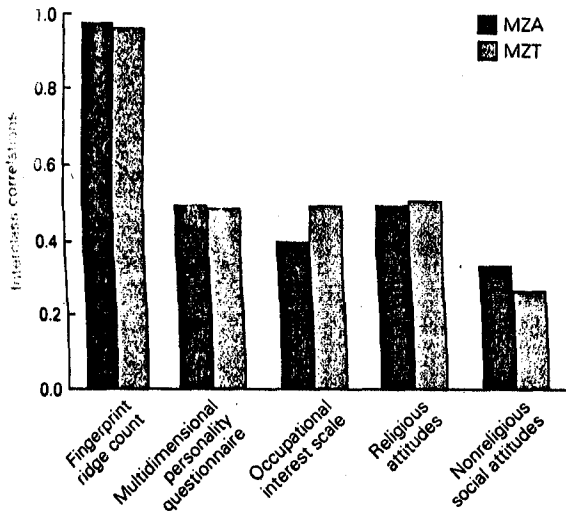
Following Galton's initial insight, genetic studies of human behavior and disease have relied heavily on the analysis of kinship. Relatives share varying degrees of genetic information and are classified as first degree (parents, siblings, and offspring), second degree (grandparents, grandchildren, nephews and nieces, half-siblings), third degree (first cousins), and so on, depending on the number of steps, more precisely the number

of generations (meiotic events), separating the members of the family tree.

Despite the uncontrolled nature of this early study, Galton was among the first to address the interplay of inheritance (nature) and environment (nurture) in the determination of behavior. Galton was well aware that relatives of eminent individuals also share social, educational, and financial advantages, and that these environmental factors might also account for the correlation between eminence and familial relationship. He therefore endeavored to assess more accurately the relative contributions of heritable and environmental factors to behavioral traits. Thus, in 1883 he introduced the idea of the twin study, a method that today remains a primary strategy for evaluating the role of genes and environment in complex behavioral traits.

Identical twins are monozygotic; they develop from a single zygote that splits into two soon after fertilization. As a result, identical twins share all genes; they are as alike genetically as is possible for two individuals. In contrast, fraternal twins are dizygotic; they develop from two different fertilized eggs. Thus, dizygotic twins, like normal siblings, share on average half their genetic information. Systematic comparisons of pairs of identical versus fraternal twins can be used to assess the importance of genes in the development of a particular trait. If identical twins tend to be more similar (concordant) than fraternal twins, the trait is attributable, at least in part, to genes.

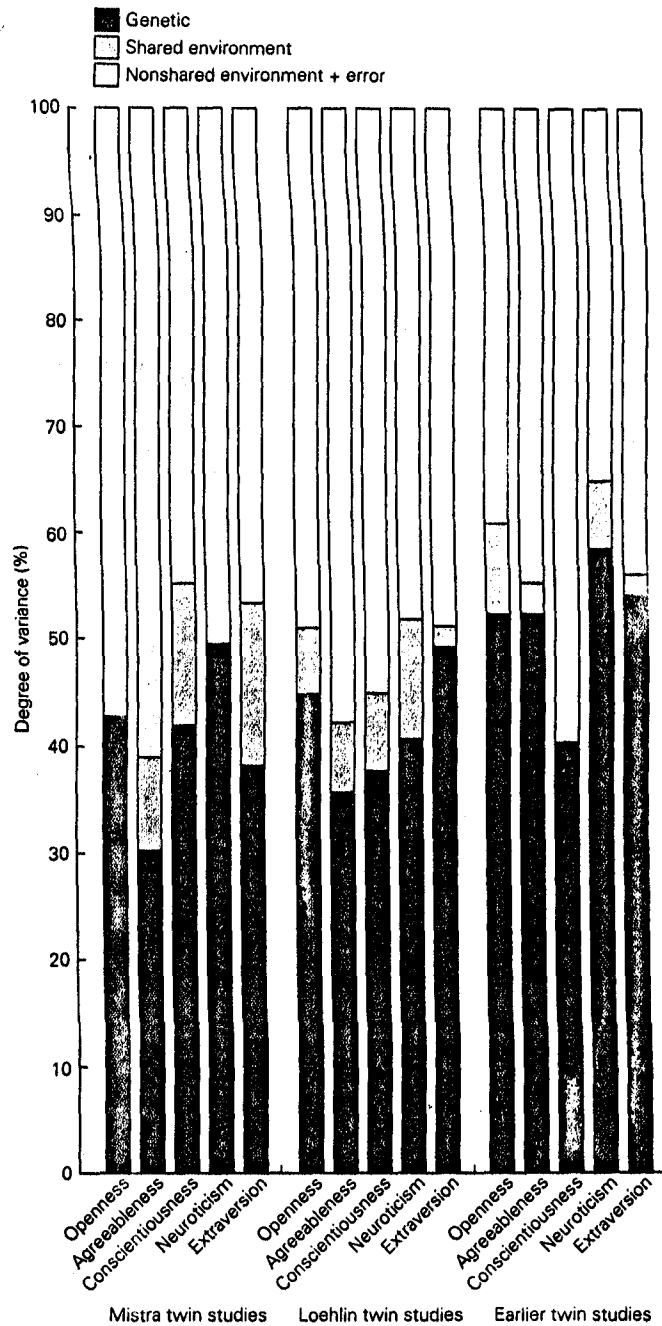
The findings from such twin studies are further sup-



**Figure 3-3** Correlations among monozygotic twins reared together (MZT) and those reared apart (MZA) for physiological characteristics, personality traits, interests, and attitudes. A score of zero represents no correlation—the average result for two random members of the population—while a score of 1.0 represents a perfect correlation. Fingerprint ridge count, which is not expected to be subject to significant environmental influence, is virtually identical in MZA and MZT pairs. Other characteristics, expected to be more subject to environmental influences, are not so highly correlated within each class. Although the correlations for these characteristics are low, the results for MZT and MZA are similar. The correlations for the multidimensional personality scale and religious attitudes among MZT and MZA are virtually identical, suggesting a significant, though not necessarily predominant, genetic influence on those traits. Correlations for the occupational interest scale and nonreligious social attitudes among MZA and MZT are more different between the two groups. (Based on Bouchard et al. 1990.)

ported by studies of identical twins that have been separated early in life and raised in different households. Despite sometimes great differences in their environment, such twins share a remarkable number of behavioral traits that we normally consider to be distinctive features of individuality, such as intellectual, religious, and vocational interests (Figures 3-3 and 3-4). Behavioral similarities between identical twins that have been separated at birth are attributable in part to genes, although environmental factors may also play a role. In general, twin studies reinforce the idea that human conduct is shaped by genetic factors but do not refute the role of environmental influences, which clearly exist.

The environmental contribution to behavioral traits is often divided into *shared* and *nonshared* components. Shared environmental influences, such as child-rearing practices or income, may underlie observed phenotypic similarities among family members. In contrast, non-



**Figure 3-4** Variation in personality in studies of twins. The units express the degree of variance accounted for by various genetic and environmental influences. (Based on Bouchard 1994.)

shared influences, such as interactions with peers in school, can create differences among members of the same family. As discussed below, similarities in personality between biological relatives are due primarily to genetic components, with differences arising from genetic factors and nonshared environmental factors.

Although studies of identical twins and kinships provide strong support for the idea that human behavior has a significant hereditary component, they do not tell us how many genes are important, let alone how specific genes affect behavior. These questions can be addressed by genetic studies in experimental animals in which both the gene and the environment are strictly controlled and by studies of human genetic mutations that give rise to diseases.

### Single Gene Alleles Can Encode Normal Behavioral Variations in Worms and Flies

A number of studies of natural populations of flies and worms have found that allelic polymorphisms in single genes can contribute to individual differences in naturally occurring behavior, including social behavior. The first example was provided by Ron Kondoka and his colleagues, who found variants in the circadian rhythm of flies as a result of molecular polymorphisms in the *period* gene. Wild-type flies vary in how well they can maintain their circadian rhythm in the presence of a temperature change, a feature called *temperature compensation*. As we will discuss below, the protein products of the *period* and *timeless* genes are involved in an autoregulatory feedback that is critical for circadian rhythms. The *per* gene has a repeat region of threonine-glycine that is polymorphic in length. Two of the major variants (with 17 repeats and 20 repeats) are found in Europe along a north-south cline. Flies with long repeats are better able to compensate for temperature shifts than those with short repeats.

A second example of such individual differences was discovered by Marta Sokolowski and her colleagues while examining the natural variation in the foraging behavior of fly larvae. Some larvae are rovers and others are sitters. Rovers follow longer foraging paths, whereas sitters use much smaller paths. The rover larvae also tend to move between patches of food, while the sitters tend to remain feeding within a food pack. This difference between rovers and sitters results from a single gene called *forager*. The rover allele has complete dominance over the sitter allele. In nature there are 70% rovers and 30% sitters. In fact, sitter larvae can be converted to rover larvae by expressing in them the gene encoding the rover phenotype. The forager gene encodes a cGMP-dependent protein kinase whose activi-

ties are higher in rover than in natural sitters, or sitter mutants, which suggests that the protein kinase may be regulated differently in the two natural variants.

Single genes can even account for differences in normal social behavior. In the course of studying 22 natural isolates of the nematode worm *Caenorhabditis elegans* collected from various locations around the world, Jonathan Hodgkin and Tabitha Doniach had found that, when grown on the surface of agar-filled Petri plates seeded with *Escherichia coli*, these natural isolates distributed themselves on the agar surface in two ways. Half the strains dispersed evenly across the bacterial patch, but the other strains spontaneously formed large, dense aggregates called *clumps*. This clumping arises, at least in part, from interaction among the worms in the clump. Mario deBono and Cornelia Bargmann realized that this reflected an example of individual differences in social behavior. They called the dispersing strains *solitary* and the clumping strains *social*.

Bargmann and deBono have identified natural variants in the behavior of worms feeding on *E. coli* in a Petri dish. Some worms are solitary foragers, moving across the food and feeding alone, while others are social foragers aggregating together on the food while they feed. More than 50 percent of the social foragers are found in groups, whereas less than two percent of the solitary foragers are found in groups. The social worms may aggregate due to the presence of a mutually attractive, as yet unidentified stimulus.

DeBono and Bargmann gathered social strains of worms that arose from mutagenesis screens of solitary strains in several laboratories and found that the mutation encodes for a gene that resembles the neuropeptide Y receptor, a G protein-coupled receptor that is ubiquitous and important in mammals for feeding. Genetic analysis of normal, wild-type strains showed that the difference between social and solitary strains was due to the substitution of a single amino acid in a cytoplasmic loop of the neuropeptide Y receptor gene. Neuropeptides are found in the brain along with conventional small molecules and are often involved in regulating responses over long periods of time. Since neuropeptide Y receptors are associated with feeding and appetite in mammals, it raises the intriguing possibility that closely related peptides might control foraging and eating behaviors in a variety of organisms that are evolutionarily divergent.

### Mutations in Single Genes Can Affect Certain Behaviors in Flies

The influence of genes on behavior can be explored most rigorously in simple animals, such as the fruit fly

## Box 3-2 Introducing Transgenes in Flies and Mice

Genes can be manipulated in mice by injecting DNA into the nucleus of newly fertilized eggs (Figure 3-5). In some of the injected eggs the new gene, or transgene, is incorporated into a random site on one of the chromosomes and, since the embryo is at the one-cell stage, the incorporated gene is replicated and ends up in all (or nearly all) of the animal's cells, including the germline.

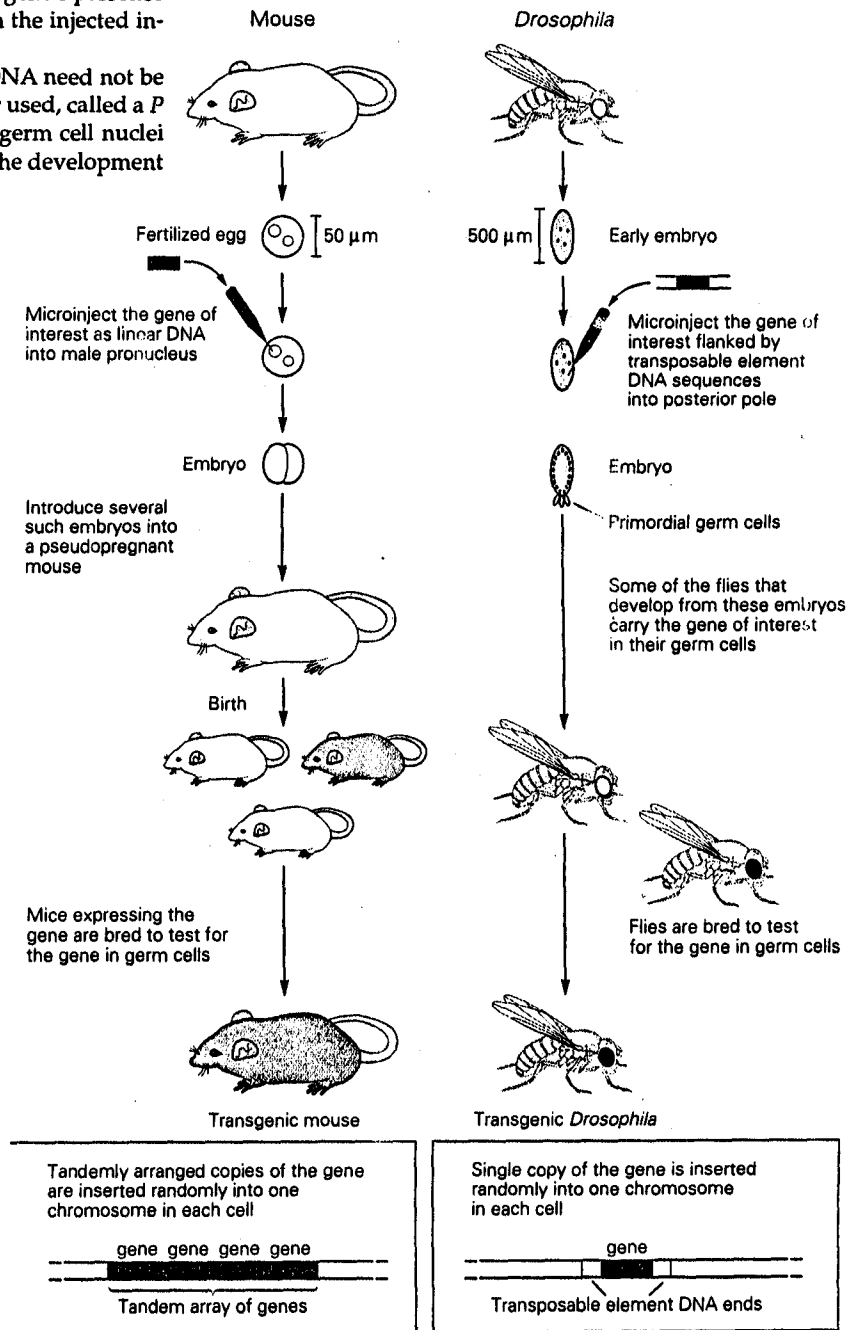
Gene incorporation is most easily detected by coinjecting the marker gene for pigment production into an egg obtained from an albino strain. Mice with patches of pigmented fur indicate successful expression of DNA. The transgene's presence is confirmed by testing a sample of DNA from the injected individuals.

A similar approach is used in flies. The DNA need not be injected directly into a nucleus since the vector used, called a *P element*, is capable of being incorporated into germ cell nuclei at the time the first cells form in the embryo. The development

and function of the nervous system of flies can be altered using promoters that are expressed ubiquitously, such as the inducible heat-shock promoter *hsp70* in *Drosophila*. More specific patterns of expression in brain cells can be obtained using promoter and enhancer sequences from genes that are specific to a cell type.

Transgenes may be wild-type genes that rescue a mutant phenotype or novel "designer" genes that drive expression of a gene in new locations or produce a specifically altered gene product.

**Figure 3-5 Standard procedures for generating transgenic mice and flies.** Here the gene injected into the mouse causes a change in coat color, while the gene injected into the fly causes a change in eye color. In some transgenic animals of both species the DNA is inserted at different chromosomal sites in different cells (see illustration at bottom). (From Alberts et al. 1994.)



*Drosophila*. Mutations of single genes in *Drosophila* can produce abnormalities in learned as well as innate behaviors, such as courtship and circadian rhythms. Moreover, mutations that affect specific aspects of behavior can readily be induced in flies (Box 3-2).

The genetic analysis of the behavior of flies has its origins in the behavioral screens performed in the 1970s by Seymour Benzer and his colleagues. These screens detected and isolated mutations that affect circadian (daily) rhythms, courtship behavior, movement, visual perception, and memory. The powerful techniques of *Drosophila* molecular genetics have enabled investigators to identify these genes and characterize how their protein products act. Here we shall focus on one class of genes isolated by Benzer, those that affect circadian rhythms. In Chapter 63 we shall consider genes in *Drosophila* that influence memory.

Many aspects of animal physiology and behavior fluctuate in rhythmic cycles. Most of these rhythms follow a circadian period; others follow shorter-term (ultradian) periods. Circadian clocks are thought to have a significant adaptive advantage. For example, they provide a means of anticipating dawn and thereby coordinate physiological functions with environmental conditions. Circadian rhythms affect everything from locomotor activity to mood and play a major role in the biology of motivation (see Chapter 51). Because of the ubiquity of these clocks among animals (and even fungi), experimental advances in invertebrates should aid in our understanding of human circadian behaviors.

Clocks have three basic features. First, the core of the clock is an intrinsic *oscillator* capable of producing a circadian periodicity of approximately 24 hours. Second, this intrinsic oscillator can adapt its rhythm to changes in the duration of the day-night cycle throughout the year. This regulation is primarily achieved through various *light-driven signals* that are transmitted by the eye to the brain, where the signals in turn act on the oscillator. Third, there are a set of *output pathways* from the oscillator that control specific behaviors, such as sleep and wakefulness and locomotor activity.

Mutations altering biological rhythms have been isolated in several organisms. The greatest insight into the oscillator has been obtained from studies of two genes in *Drosophila*, the *period (per)* gene, identified originally by Benzer and his colleagues, and the *timeless (tim)* gene identified recently. The *period* and *timeless* genes appear to be devoted almost exclusively to the control of rhythms. Even when they are eliminated, the organism has no other major defects.

Mutations in either the *per* or *tim* gene affect the circadian rhythms of locomotor activity and eclosion (i.e., the emergence of the adult from the pupa). Arrhythmic

*per* mutants exhibit no discernible rhythms in either of these behaviors. A long-day *per* allele produces 28-hour cycles for both locomotor activity and eclosion, whereas two short-day *per* alleles shorten the cycle (to 19 hours in one case and to 16 hours in the other; see Figure 3-6).

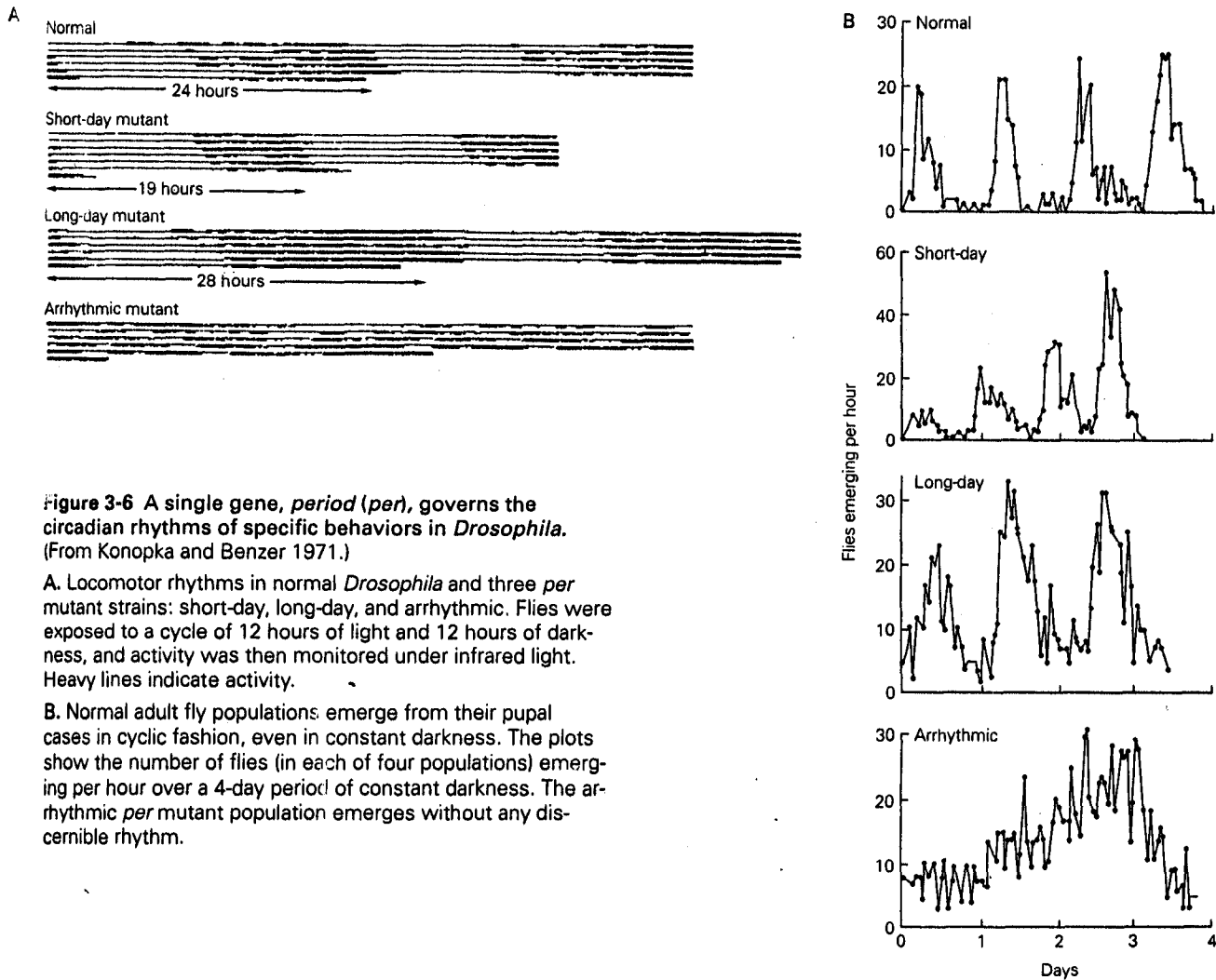
How do the *per* and *tim* genes keep time? The answer to this question has begun to emerge from genetic and molecular studies of the two genes and their protein products. The protein products of the *per* and *tim* genes (PER and TIM) are thought to shuttle between the cytoplasm and nucleus of cells, regulating expression of target genes, including themselves. As a result, the synthesis and accumulation of the messenger RNAs encoding PER and TIM follow a circadian cycle.

For the proteins to function, PER has to bind to TIM (Figure 3-7). Both genes are transcribed in the morning and their mRNAs accumulate during the day, during which the protein products appear not to be functional. A key step in the regulation of this cycle is the light-induced degradation of the TIM protein. During the day *tim* RNA is transcribed but the level of TIM protein remains low because of a high rate of degradation. In the absence of TIM, PER does not function. As a result, TIM and PER complexes are not formed. After dusk, when the levels of TIM and PER increase, the two proteins bind to one another, thus becoming functional, and enter the nucleus where they inhibit the transcription of their own genes as well as other, unidentified target genes. As a consequence, *per* and *tim* mRNA levels decrease and subsequently protein expression decreases. By morning, PER and TIM protein levels have fallen to low enough levels that they no longer repress transcription.

The finding that the *per* and *tim* transcripts are regulated by negative feedback raises the question of why the PER and TIM proteins do not immediately repress their own expression. The answer lies in a built-in delay in accumulation and translocation of the proteins to the nucleus. The PER protein cannot accumulate until sufficient TIM protein is present to bind to and stabilize it. TIM protein, on the other hand, cannot enter the nucleus unless it is bound to PER protein. Accurate time-keeping therefore depends on an oscillatory cycle in gene expression and inactivation by negative feedback.

What does this say for mechanisms in normal and short-cycle flies? In the long-day (28-hour) *per* mutants the binding affinity of PER proteins for TIM appears to be reduced. Binding thus cannot occur until the two proteins reach higher levels, causing a delay in the entry of the PER-TIM complex into the nucleus and thus extending the period of each cycle.

The mechanisms that control circadian rhythms in other organisms are likely to be similar in principle to



**Figure 3-6** A single gene, *period (per)*, governs the circadian rhythms of specific behaviors in *Drosophila*. (From Konopka and Benzer 1971.)

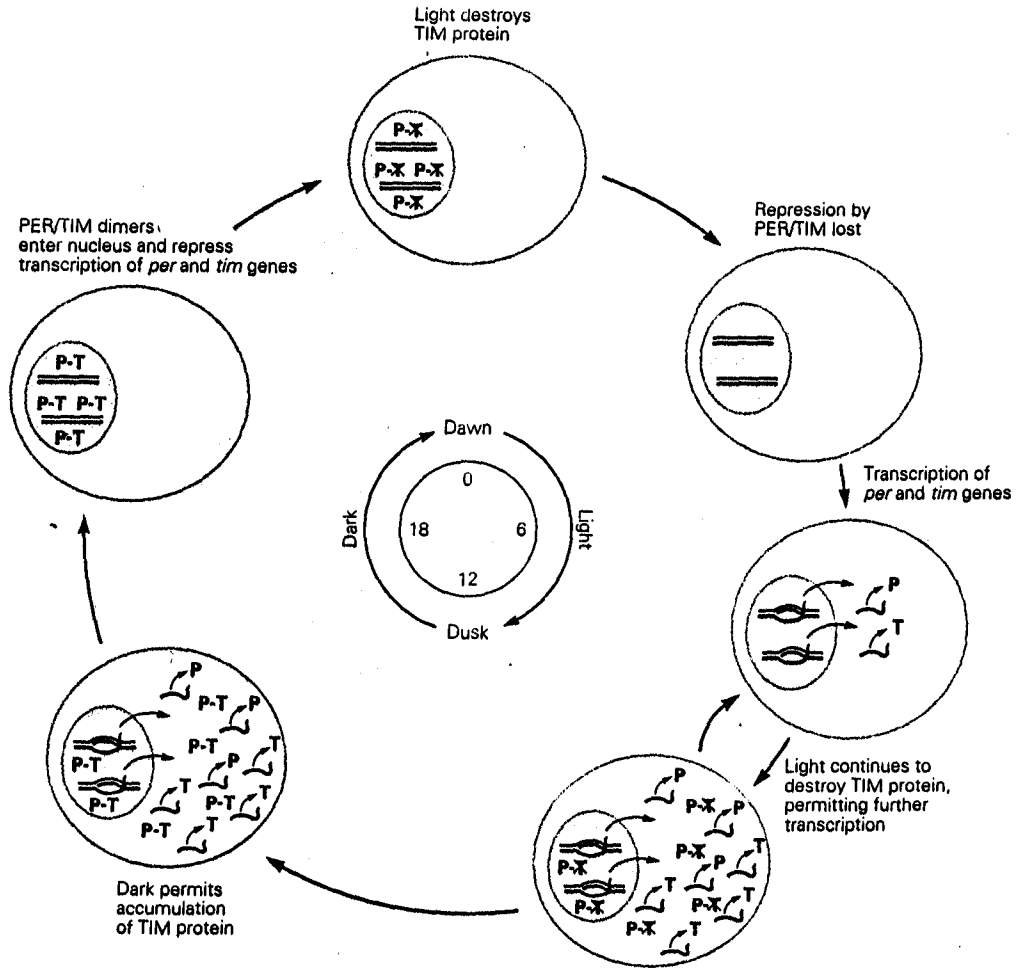
**A.** Locomotor rhythms in normal *Drosophila* and three *per* mutant strains: short-day, long-day, and arrhythmic. Flies were exposed to a cycle of 12 hours of light and 12 hours of darkness, and activity was then monitored under infrared light. Heavy lines indicate activity.

**B.** Normal adult fly populations emerge from their pupal cases in cyclic fashion, even in constant darkness. The plots show the number of flies (in each of four populations) emerging per hour over a 4-day period of constant darkness. The arrhythmic *per* mutant population emerges without any discernible rhythm.

the mechanism that controls the rhythmicity of the *per* and *tim* genes in *Drosophila*. In mammals circadian behavioral rhythms are governed by the suprachiasmatic nucleus in the hypothalamus (see Chapter 47). Because circadian behavior in mice is precise, it is easy to set up quantitative genetic screens for mutations that alter the circadian behavior. Joseph Takahashi took advantage of the regularity of this behavior to carry out a chemical mutagenesis screen. By this means he identified a semi-dominant autosomal mutation named *clock*. Mice homozygous for the *clock* mutation show extremely long circadian periods followed by a complete loss of circadian rhythmicity when transferred to constant darkness (Figure 3-8). The *clock* gene therefore appears to regulate two fundamental properties of the circadian rhythm in

mice: the circadian period itself and the persistence of circadian rhythmicity.

Since no anatomical defects have been observed with the *clock* mutation, the *clock* gene appears to encode a protein specific and essential for circadian rhythmicity in the mouse. When the *clock* gene was cloned it was found to encode a transcription factor, presumably involved in the basic regulation of genes important for the circadian rhythm. Particularly important is the fact that one of the domains of the clock protein (the PAS domain) is also found in PER. This raises the interesting possibility that the clock protein might bind to and interact with a mouse protein homologous to PER. Many mammalian genes related to *clock* have now been identified and implicated in the control of circadian rhythms.



**Figure 3-7** Light-dependent degradation of the TIM protein establishes the circadian control of biological rhythms in *Drosophila*. The genes that control the circadian clock are regulated by two nuclear proteins, PER and TIM, that slowly accumulate and then bind to one another to form dimers. Dimerization of PER and TIM is necessary for the complex to enter the nucleus and shut off the transcription of target genes, including the genes for PER and TIM themselves. During the

hours of daylight TIM protein is degraded by light; thus PER cannot enter the nucleus and the transcription of target genes (including the *per* and *tim* genes) continues. After dark, TIM protein is no longer degraded, and the PER-TIM dimers enter the nucleus, where they repress transcription of target genes. In this way the day-night cycle regulates the expression of genes that control biological function. (Adapted from Barinaga 1996.)

## Defects in Single Genes Can Have Profound Effects on Complex Behaviors in Mice

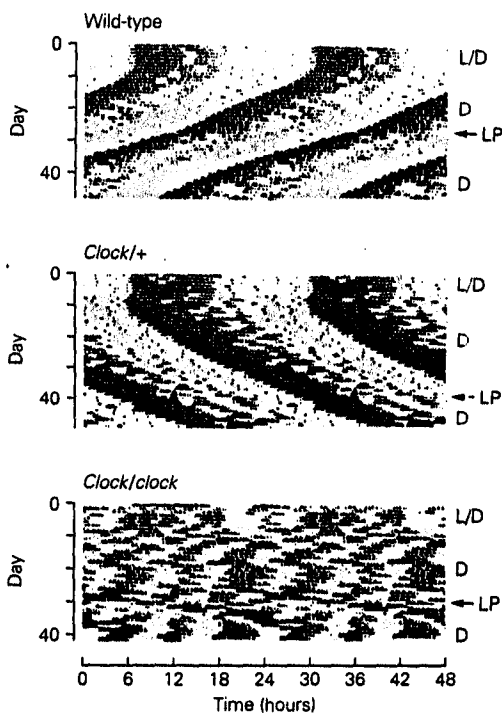
The use of chemical genetic techniques to identify circadian rhythm mutants in mice underscores the importance of this experimental mammal in behavioral genetic studies. Genetic studies of mouse behavior have begun to provide insight into the genetic bases of some human behavioral disorders. Here we discuss the evidence for a genetic basis for three disorders: obesity, impulsivity, and altered motivational state.

### Mutations in the Gene Encoding Leptin Affect Feeding Behavior

Whether an individual is lean, obese, or of intermediate size is determined in large part by the balance between the amount of food consumed and energy expended, a balance governed by both psychological and physiological factors. Genetics studies of obese mice have provided the best insight into the physiological factors that control ingestive behavior.

The physical cloning and characterization of the region around a spontaneous obesity-causing mutation on mouse chromosome 6 led to the identification of the mouse *obese* (*ob*) gene and to a highly conserved (homologous) human gene. The mouse *ob* gene encodes the protein leptin, a small protein of 145 amino acids that is selectively expressed in adipose tissue and released into the bloodstream. Leptin contributes to the homeostatic mechanisms that permit an animal to maintain its weight within 5% of its normal weight for most of its life. Under normal conditions the amount of leptin secreted reflects the total mass of adipose tissue. When adipose tissue decreases, leptin levels decrease and the animal eats more; when adipose tissue increases, leptin levels increase and the animal eats less. Mice with homozygous mutations in the *ob* gene lack circulating leptin. This lack leads to marked obesity in these mutant animals. When leptin is supplied exogenously, however, food intake and body weight are reduced dramatically.

A receptor for leptin, called OB-R, encodes a protein that is related to a component of certain cytokine receptors that activate specific transcription factors. This leptin receptor is expressed at a high level in the hypothalamus, the part of the brain that controls appetite and feeding (Chapter 32). The gene encoding OB-R is located in the same region of mouse chromosome as the *diabetic* gene (*db*). This is interesting because obesity and diabetes are often linked in humans. In fact, *db/db* mice are also obese and exhibit a phenotype similar to the mice with a mutated *ob* gene. Moreover, there is good



**Figure 3-8** Locomotor activity records of *clock* mutant mice. The record shows periods of wheel-running activity by three offspring. All animals were kept on a light-dark cycle (L/D) of 12 hours for the first 7 days, then transferred to constant darkness (D). They later received a 6-hour light pulse (LP) to reset the rhythm: The activity rhythm for the wild-type mouse had a period of 23.1 hours. The period for the heterozygous *clock/+* mouse is 24.9 hours. The homozygous *clock/clock* mice experience a complete loss of circadian rhythmicity upon transfer to constant darkness and transiently express a rhythm of 28.4 hours after the light pulse. (From Takahashi et al. 1994.)

evidence that the *db* gene encodes the leptin receptor.

To what extent do these studies of mice provide insight into human disease? Most obese humans are not defective in leptin mRNA or protein levels and indeed produce higher levels than do nonobese individuals. Thus, it is likely that human obesity reflects not a lack of leptin but a failure to respond to normal or even elevated levels of leptin. Failure to respond to leptin could be a result of mutations of the leptin receptor or of molecules that interact with the receptor.

Leptin may affect feeding behavior by regulating neuropeptide and neurotransmitter expression in hypothalamic cells. Lesions of the hypothalamus affect body weight. For example, ablation of the ventromedial hypothalamus or the arcuate nucleus results in obesity. Leptin administration markedly inhibits the biosynthesis and release of neuropeptide Y, a peptide that stimulates food

**Flies**

Genetic analysis of behavior in *Drosophila* relies on behavioral assays of animals in which individual genes have been mutated. Experimental mutations in *Drosophila* were originally produced through radiation-induced mutagenesis. This method, however, results in large-scale deletions or rearrangements in chromosomes; several genes are often affected, even when small deletions are the target, and molecular characterization of relevant genes is difficult. In contrast, the chemical ethyl methanesulfonate (EMS) induces point mutations and thus facilitates the characterization of mutations at specific loci.

Many spontaneous mutations and chromosomal rearrangements are produced by transposable elements. The most useful class of transposable elements in *Drosophila* is the P element. P elements encode a transposase enzyme that mediates the mobilization of the element and a repressor product that blocks transposition. P elements have become major tools of the modern *Drosophila* geneticist.

In one technique, P elements are used to isolate mutations in any *Drosophila* gene of interest. The investigator screens for mutants of the gene in progeny of crosses between *Drosophila* strains that carry P elements and those in which they are absent. New mutations result from the transposition of a P element into a gene. A vector is then constructed in which a P element is inserted. This vector is used as a probe to identify and

isolate DNA segments that contain P elements; elements inserted into the gene of interest are found within a subset of these segments. The gene can then be cloned and studied.

**Mice**

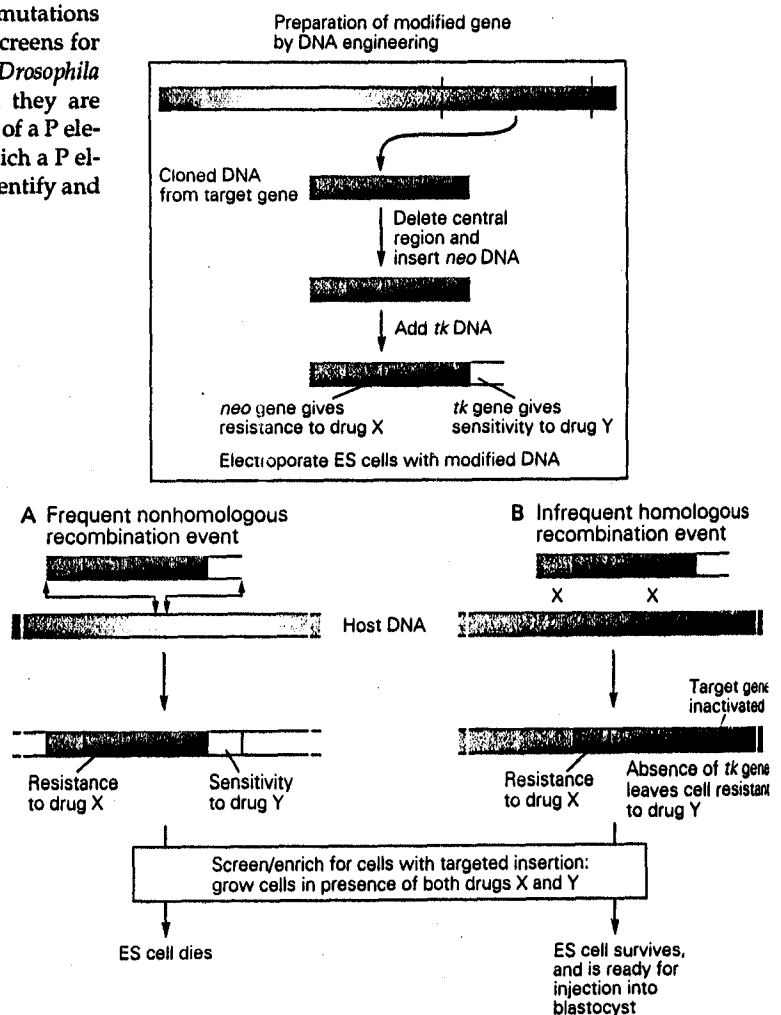
Recent advances in molecular manipulation of mammalian genes have permitted *in situ* replacement of a known, normal gene with a mutant version. The process of generating a strain of mutant mice involves two separate manipulations: the replacement of a gene on a chromosome by homologous recombination in a special cell line known as embryonic stem cells (Figure 3-9), and the subsequent incorporation of this modified cell line into the germ cell population of the embryo (Figure 3-10).

The gene of interest must first be cloned. The gene is mutated, and a selectable marker, usually a drug-resistance gene,

**Figure 3-9.** Experimentally controlled homologous recombination is the first step in creating mutant mice. Cloned DNA from the mouse gene to be mutated is modified by genetic engineering so that it contains a bacterial gene, *neo*. Integration of *neo* into a mouse chromosome makes the mouse cells resistant to drugs that otherwise would be lethal to the cells (drug X). A viral gene, *tk*, is also added, attached to one end of the mouse DNA. Integration of *tk* into a mouse chromosome makes the cells sensitive to a different drug (drug Y). (Adapted from Alberts et al. 1994.)

**A.** Most insertions occur at random sites in the mouse chromosome, and these nearly always include both ends of the engineered DNA fragment. Colonies of cells in which homologous recombination has incorporated the center of the engineered DNA fragment without the ends are obtained by selecting for those rare mouse cells that grow in the presence of both drugs.

**B.** Most of the cells that grow in the presence of both drugs will carry the targeted gene replacement.

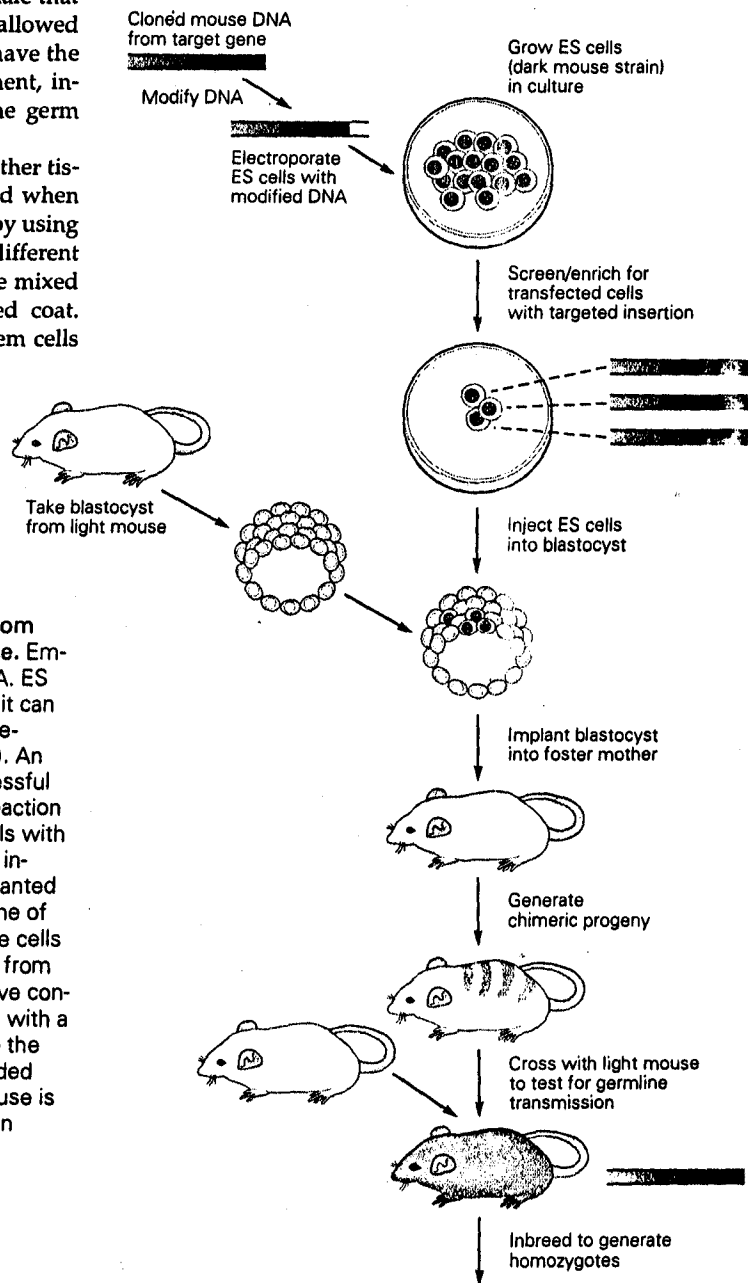


is then introduced into the mutated fragment. The altered gene is then transfected into embryonic stem cells, and clones of cells that incorporate the altered gene are isolated. To identify a clone in which the mutated gene has been integrated into the homologous (normal) site, rather than some other random site, DNA samples of each clone are tested.

When a suitable clone has been obtained, cells are injected into a mouse embryo at the blastocyst stage (3–4 days after fertilization), when the embryo consists of approximately 100 cells. These embryos are then reintroduced into a female that has been hormonally prepared for implantation and allowed to come to term. Embryonic stem cells in the mouse have the capability of participating in all aspects of development, including the germline. Thus, injected cells can become germ cells and pass on the altered gene.

Since incorporated stem cells generally mix into other tissues besides the germline, their presence can be tested when the injected embryo is born. Initially, this can be done by using a stem cell line from a mouse strain with a fur color different from that of the strain used to obtain the embryo. The mixed (chimeric) offspring appear to have a patchy colored coat. These progeny are then mated to determine if any stem cells

have become germ cells. If so, their progeny will carry the altered gene on one of their chromosomes, detectable by analyzing DNA samples from each of the offspring. When the heterozygous individuals are mated together, one-fourth of the progeny will be homozygous mutant. This technique has been used to generate mutations in various genes crucial to development or function in the nervous system.



**Figure 3-10. Altered embryonic stem cells derived from mouse blastocysts are used to create transgenic mice.** Embryonic stem (ES) cells are transfected with altered DNA. ES cells that have integrated a transgene for a particular trait can be selected by using a donor that carries an additional sequence, such as a drug-resistance gene (see Figure 3-9). An alternative is to assay the transfected ES cells for successful integration of the donor DNA using polymerase chain reaction (PCR) technology. After obtaining a population of ES cells with a high proportion carrying the marker, the cells are then injected into a recipient blastocyst. This blastocyst is implanted into a foster mother to generate a chimeric mouse. Some of the tissues of the chimeric mice will be derived from the cells of the recipient blastocyst; other tissues will be derived from the injected ES cells. To determine whether ES cells have contributed to the germline, the chimeric mouse is crossed with a mouse that lacks the donor trait. Any progeny that have the trait must be derived from germ cells that have descended from the injected ES cells. By this means, an entire mouse is generated from the altered ES cell. (Adapted from Lewin 1994.)

intake when administered to rodents. Remarkably, as we have discussed earlier, the link between neuropeptide Y and food intake appears to have been conserved, in a general sense, between *C. elegans* and man.

### Mutations in the Gene Encoding a Serotonergic Receptor Intensify Impulsive Behavior

Serotonin (5-hydroxytryptamine) is a monoamine that serves as a neurotransmitter in the brain. The level of serotonin is thought to be reduced in depressive illness. As we shall learn later (Chapter 44), neurons that synthesize serotonin are clustered in several nuclei in the brain stem, the most prominent of which are the *raphe nuclei*. Their axons project to many regions of the brain, notably the cerebral cortex. Neurons that synthesize serotonin modulate the activity of cortical and subcortical neurons in several ways by activating different receptor subtypes: some excitatory, some inhibitory, some both.

Because of its action on different receptors, serotonin has been implicated in the regulation of mood states, including depression, anxiety, food intake, and impulsive violence (see Chapter 61). Several animal studies have shown that aggressive behavior is often associated with decreased activity of serotonergic neurons. These studies are of particular interest because they provide a glimpse of how social and genetic factors interact to modify behavior.

Most animals, including humans, become aggressive when threatened, such as when their territory is invaded, their offspring are attacked, or sexual interactions are prevented. The importance of serotonergic transmission in aggressive behavior is clearly evident in studies of mice in which the gene for the serotonin 1B receptor has been ablated by targeted deletion (Box 3-3). When mice lacking the serotonin 1B receptor are isolated for four weeks and then exposed to a wild-type mouse, they are much more aggressive than wild-type animals under similar conditions. The mutant mice attack intruders faster than wild-type mice or mice lacking only one copy of the serotonin 1B receptor gene, and the number and intensity of attacks is significantly greater than that of wild-type mice. Thus, the serotonin 1B receptor plays a role in mediating aggressive behavior in mice.

Serotonin activity has been implicated as one of several important biological factors in determining the threshold for violence. People with a history of impulsive aggressive behavior (and of suicide)—and mouse strains that display increased aggressiveness—have low concentrations of serotonin in the brain. Inhibition of serotonin synthesis or destruction of serotonergic neu-

rons increases aggressiveness in mice and monkeys. Finally, certain serotonin agonists that act on the serotonin 1B receptor inhibit aggression.

In humans a variety of social stressors, such as social or sexual abuse during childhood, are thought to lower the biological thresholds for violence, including the level of serotonin in the brain. Indeed, male monkeys raised in isolation have reduced levels of serotonin in their brains, illustrating that both environmental and genetic factors can converge to influence the metabolism of serotonin.

The relationship of serotonin levels to aggression in humans is not simple, however. This complexity is evident in studies of a Dutch family that transmits an X-linked form of mental retardation. Fourteen of the affected males have a history of impulsive behavior that includes arson, rape, and attempted murder. Each of these men carries a point mutation in the gene that encodes the enzyme monoamine oxidase A, one of the two major enzymes that metabolizes monoamines. This class of neurotransmitter includes serotonin, norepinephrine, and dopamine (see Chapters 60 and 61). The mutation apparently leads to *increased* levels of serotonin, yet the affected people show enhanced impulsiveness. Thus, the relationship between serotonin and aggression is not simply that reduced serotonin causes aggression and enhanced serotonin causes placidity. Both increases *and* decreases in serotonin levels may enhance aggression. These findings suggest, not surprisingly, that in humans the relationship between serotonin and a complex trait such as aggression is not direct and may be quite subtle. Finally, although monoamines, in particular serotonin, are important in aggressive behavior, other transmitter systems also affect this behavior, as would be expected for a complex behavioral trait.

### Deletion of a Gene That Encodes an Enzyme Important for Dopamine Production Disrupts Locomotor Behavior and Motivation

Dopamine, like serotonin, is a major monoaminergic transmitter in the central nervous system. The majority of dopaminergic neurons have their cell bodies in the substantia nigra while their axons project to the corpus striatum. Dopaminergic neurons have been implicated in the regulation of motor behavior—the degeneration of dopaminergic neurons underlies Parkinson's disease, a debilitating disorder of movement. Other dopaminergic pathways are thought to regulate motivated behaviors. Dysfunction of these pathways may contribute to schizophrenia (see Chapter 60).

The role of the dopaminergic system in mammalian

behavior has traditionally been studied through pharmacological techniques. Recently, however, gene knockout techniques have been applied to this system. In one set of experiments the ability of neurons to synthesize dopamine was blocked by selectively inactivating the gene that encodes tyrosine hydroxylase, one of the enzymes important in dopamine synthesis. The dopamine-deficient mice were born, began to nurse, and grew normally for about two weeks and then became inactive, failed to eat or drink, and died shortly thereafter. However, daily administration of L-DOPA, the product of tyrosine hydroxylase, restored normal feeding and produced increased activity.

Dopamine is cleared from the synapse by a high-affinity dopamine transporter. In mutant mice with a deficiency in this transporter the amount of extracellular dopamine is 100-fold greater than normal. The mutant mice exhibit spontaneous and excessive locomotion similar to that obtained in normal mice when the dopamine transporter is blocked pharmacologically (as with a psychostimulant such as cocaine).

### Single Genes Are Critical Factors in Certain Human Behavioral Traits

#### Mutations in a Dopamine Receptor May Influence Novelty-Seeking Behavior

As we have seen, studies of identical twins suggest that a number of personality characteristics have a significant heritable component, but in no case has this finding been rigorously demonstrated by identifying a specific gene. One fascinating candidate is novelty-seeking behavior, a behavior characterized by exhilaration or excitement in response to stimuli that are novel. People who score high on tests of novelty seeking tend to be impulsive, exploratory, fickle, excitable, quick-tempered, and extravagant. They often do things for thrills, as opposed to thinking things through before coming to a decision.

Twin studies suggest that novelty-seeking behavior has a heritability of about 40%. A significant component (10% of the genetic component) seems to be due to a polymorphism in a single gene, the gene that encodes the D4 dopamine receptor. Dopamine is involved in exploratory and pleasure-seeking behavior. There are at least five known receptors for dopamine, called D1 to D5 (Chapter 60). The D4 receptor is expressed in the hypothalamus and the limbic areas of the brain concerned with emotion.

In general, the coding sequence of the receptors for dopamine are highly conserved (as are the coding sequence for other receptors to chemical transmitters), and polymorphisms are very rare. Nevertheless, an in-

teresting polymorphism has been found in the D4 receptor. One form of the gene, called the short form, has a 48-base pair DNA sequence in one of its cytoplasmic domains. By contrast, the long form of the D4 receptor gene has seven repeats of this domain. Additionally, the long and short forms of the receptor appear to have slightly different signaling properties in response to dopamine. It appears that these slight differences in the long form of the receptor correlate with novelty seeking.

#### Mutations in Opsin Genes Influence Color Perception

Color vision is one of the few cases in which variation in normal human perception can be explained at a molecular level. Molecular cloning techniques have been used to identify and clone the genes encoding the proteins for the red, green, and blue pigments that transduce different wavelengths of light (see Chapter 29). Defects in one or more of the genes encoding red and green pigments lead to varying degrees of color blindness.

The genes for red and green pigments are arrayed head-to-tail, close to one another on the X chromosome and differ in only about 1 in 20 of their amino acid residues. Because of this tandem organization and similarity of sequence, crossing over between the red and green pigment genes occurs frequently, leading to gene rearrangement.<sup>2</sup> The resulting abnormality in both genes explains the origin of many cases of red-green color blindness.

Subtle variations in color perception occur even among individuals with normal color vision. This is attributable to polymorphism in the red pigment gene in humans. In 62% of the male population with normal color vision, amino acid 180 is a serine residue while in the remaining 38% it is an alanine residue. The effects of this sequence difference can be revealed in psychophysical tests in which subjects are asked to match the intensity of a mixture of red and green light. The intensity of red light needed to match a standard depends on the amino acid at position 180. Because females have two X chromosomes, they fall into three groups: homozygotes for Ser180, homozygotes for Ala180, and heterozygotes who display an intermediate phenotype. Thus, a major variation in human color perception can be explained by a small change in the coding sequence of a single gene.

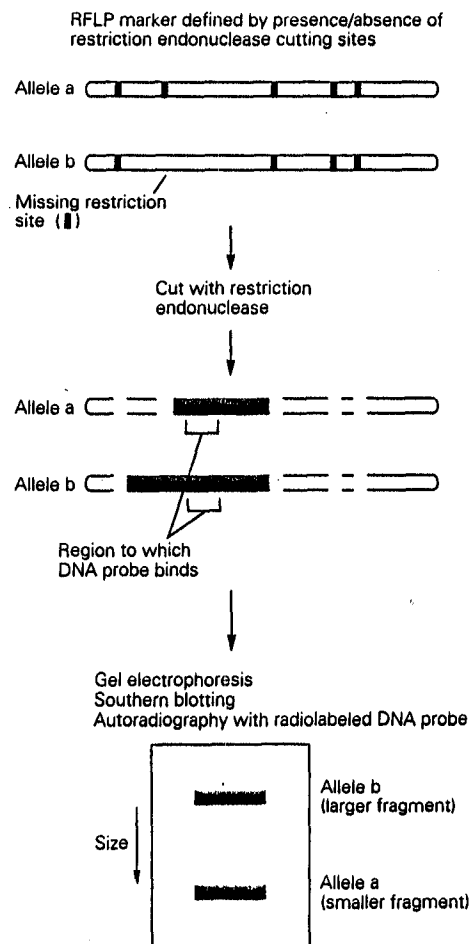
<sup>2</sup>This gene rearrangement is the result of unequal crossing over between the X chromosomes in a female. This unequal crossover appears as a *hemizygous* condition in male offspring (genes on the male's X chromosome are called hemizygous because they exist only in one copy).

### Box 3-4 Genetic Polymorphisms

If two genes are located very near one another they are likely to be inherited together. Thus, if an abnormality of one gene produces a disease and a nearby marker gene encodes a readily recognized phenotypic trait (such as hair or eye color) or a readily detectable gene product (such as a protein present in the blood), people who express the marker will likely also express the disease—even though the marker may have nothing to do with the disease. Both the phenotypic trait and the DNA sequence of the gene vary in the normal population.

In the past, genetic markers were used to distinguish variations in the protein coding regions of genes, such as blood group antigens, enzymes, and antigens of the histocompatibility complex. However, coding sequences represent only 5–10% of the total human genome; 90 or 95% of the genome contains noncoding regions. It is now possible to saturate the human genome with markers that distinguish variations that occur in otherwise homologous DNA sequences throughout the whole genome (including noncoding as well as coding sequences). This broad coverage has made it much easier to trace the inheritance of a disease to a specific region of a particular chromosome.

**Figure 3-11A.** The presence of a restriction fragment length polymorphism (RFLP) can be detected by analyzing DNA fragments cleaved by restriction endonucleases, enzymes that cut at specific restriction sites in nucleotide sequences. In this example chromosome *b* is missing a restriction site that is present on chromosome *a*. As a result, cutting chromosome *b* produces a larger than normal DNA fragment in this region. After cutting, the DNA from both chromosomes is separated according to size by means of gel electrophoresis and transferred to nylon filters (in a procedure called Southern blotting). Autoradiography is then used to reveal the polymorphism. Because the *b* fragment is larger, it is distinguishable from the *a* fragment. (Adapted from Alberts et al. 1994.)



Novelty seeking is a natural variation in human behavior. Color blindness is a similar variation in perception. It may be annoying to those who have it, but it interferes only marginally with life's function and not at all with longevity. These relatively neutral mutations differ importantly from mutations that produce serious disease.

#### Mutations in the Huntingtin Gene Result in Huntington Disease

One of the first complex human behavioral abnormalities to be traced to a single gene is Huntington disease, a

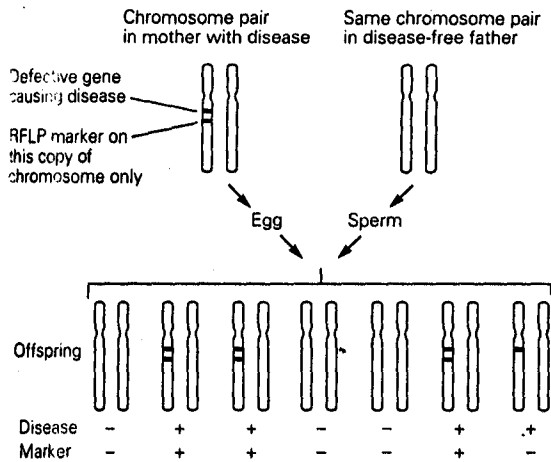
degenerative disorder of the nervous system. Huntington disease affects both men and women with a frequency of about 5 per 100,000. It is characterized by four features: heritability, chorea (incessant, rapid, jerky movements), cognitive impairment (dementia), and death 15 to 20 years after the onset of symptoms. In most patients the onset of the disease occurs in the fourth to fifth decade of life. Thus, the disease often strikes after individuals have married and had children.

Huntington disease involves the death of neurons in the caudate nucleus, a part of the basal ganglia involved in regulating voluntary movement. The death of

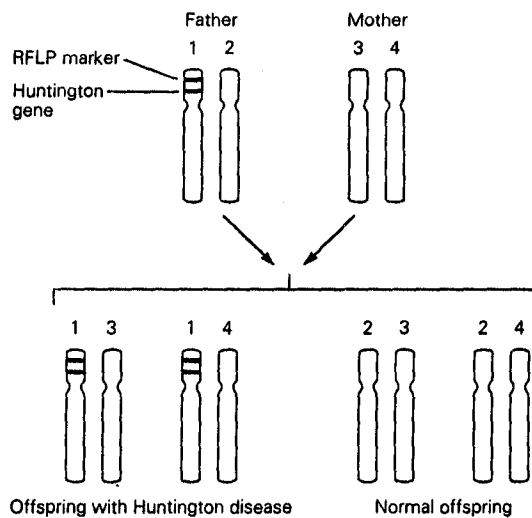
One type of DNA marker, a *restriction fragment length polymorphism* (RFLP), is created by differences in DNA sequence in paired alleles. At one allele a cutting site for a particular restriction enzyme (an enzyme that cuts DNA only at a specific nucleotide sequence) is eliminated or an extra site added, while the other allele remains normal. As a result, the restriction enzyme produces DNA fragments of different lengths from the two alleles. These so-called restriction fragments can

be separated by electrophoresis in agarose gels and distinguished by specific DNA probes (Figure 3-11A).

When such a polymorphic region of the DNA is closely linked to a particular gene, inheritance of the gene can be traced by following the inheritance of a particular pattern of restriction fragments. The method can be used to trace pathogenic genes (Figure 3-11B,C).



**Figure 3-11B.** Genetic linkage analysis detects the coinheritance of a mutated gene responsible for a human disease and a nearby restriction fragment length polymorphism (RFLP) marker. In this example the gene responsible for the disease is inherited in four offspring, three of which coinherit the marker. Thus, the gene responsible for the disease is located close to the RFLP marker on this chromosome. (Adapted from Alberts et al. 1994.)



**Figure 3-11C.** Inheritance of the gene responsible for Huntington disease can be traced by following the inheritance of a particular restriction fragment length on chromosome 4.

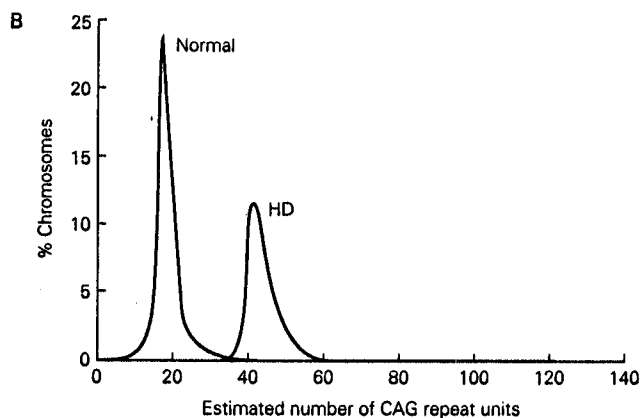
nerve cells in the caudate nucleus is thought to cause the chorea. The basis for the impaired cognitive functions and eventual dementia is less clear and is due either to a loss of cortical neurons or to the disruption of normal activity in the cognitive portion of the basal ganglia (see Chapter 43). The selective loss of neurons in the caudate nucleus can be demonstrated in living patients using imaging techniques.

Huntington disease is inherited as an autosomal dominant disorder and the mutation is highly pene-

trant.<sup>3</sup> The Huntington disease gene was identified on chromosome 4 using a technique based on DNA markers to map heritable disease mutations relative to genetic polymorphisms (Box 3-4). This gene encodes a large protein called Huntingtin, the function of which is as yet unknown.

<sup>3</sup>Penetrance refers to the frequency with which a heritable trait is manifested phenotypically by individuals carrying the mutant gene(s). Thus the Huntington disease gene is 100% penetrant.

A CGATACGTCGAT . . . . CAGCAGCAG(CAG)<sub>20</sub> . . . . . TATCTAGA Healthy  
 CGATACGTCGAT . . . . CAGCAGCAGCAG(CAG)<sub>46</sub> . . . . TATCT Huntington disease (HD)  
 Flanking sequence Repeat region Flanking sequence

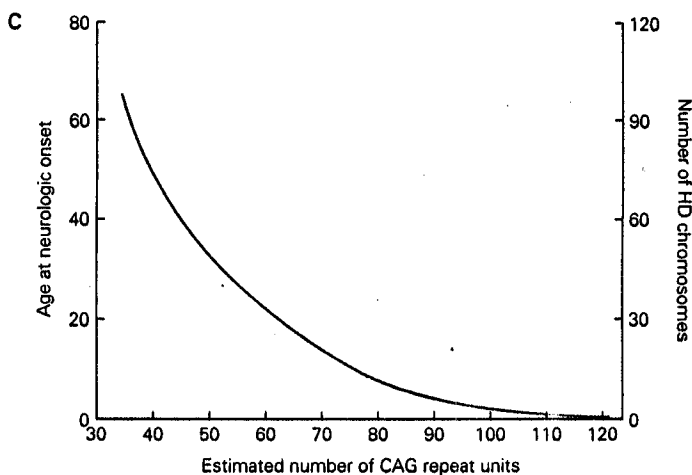


**Figure 3-12.** The DNA mutation in Huntington disease is an unstable CAG repeat.

A. The nucleotide sequence in the region of the unstable CAG repeat in the Huntingtin gene.

B. Distribution of CAG repeat lengths on normal and Huntington disease (HD) chromosomes. The percentages of normal and HD chromosomes containing different CAG repeat lengths (from 6 to 125) are compiled from several published studies.

C. A highly significant inverse correlation between age of onset of Huntington disease movements and CAG repeat length occurs across all HD alleles. (Modified from Gusella and MacDonald 1995.)



The mutated form of the Huntingtin protein contains a stretch of glutamine residues that is much longer than in the normal protein. The codon (CAG) that encodes glutamine is repeated 19–22 times in the normal gene but 48 or more times in the mutated gene (Figure 3-12A). This expansion results in abnormally long stretches of polyglutamine in the protein. The number of ways in which the abnormal stretch of glutamines affect protein function is not known.

Diseases that involve trinucleotide expansion have an additional feature: each successive generation of a family that harbors the mutant gene manifests the disease with greater severity at an earlier age (*genetic anticipation*). Thus, an individual may have a mild case of Huntington disease that was not manifested until age 60, whereas his great grandchild may develop more

serious symptoms by age 40 (Figure 3-12B,C). This trend is due to the instability of the expanded trinucleotide repeat. As the repeat passes through the germline, the number of repeats tends to increase, particularly in the paternal line. These repeats are thought to create hairpin-like structures in DNA that interfere with its replication. As the repeats attain a certain length, the hairpin-like structures stabilize, leading to persistent mistakes in replication and consequently further expansion of the trinucleotide repeat. The polyglutamine structures appear to affect the protein in one of two ways: they may make the altered protein destructive to the cell, producing a gain-of-function mutation, or they may bind other proteins required for normal cellular function. Expanded tri-nucleotide repeat diseases are usually genetically dominant.

Table 3-1 Neurological Diseases Involving Trinucleotide Repeats<sup>1</sup>

Disease	Repeat	Repeat length <sup>2</sup>	Gene product
X-linked spinal and bulbar muscular atrophy	CAG	Normal: 11–34 Disease: 40–62	Androgen receptor
Fragile X mental retardation <sup>3</sup>	CGG	Normal: 6 to ~50 Premutation: 52–200 Disease: 200 to >1000	FMR-1 protein
Myotonic dystrophy <sup>3</sup>	CTG	Normal: 5–30 Premutation: 42–180 Disease: 200 to >1000	Myotonin protein kinase
Huntington disease	CAG	Normal: 11–34 Disease: 37–121	Huntingtin
Spinocerebellar ataxia type 1	CAG	Normal: 19–36 Disease: 43–81	Ataxin-1
FRAXE mental retardation <sup>3</sup>	GCC	Normal: 6–25 Disease: >200	?
Dentatorubral-pallidoluyasian atrophy	CAG	Normal: 7–23 Disease: 49–75	?

<sup>1</sup>Eight diseases are now associated with the expansion of a trinucleotide CAG repeat in the coding region of the responsible gene: spinal and bulbar muscular atrophy (SBMA); Huntington disease (HD); dentatorubralpallidoluyasian atrophy (DRPLA); spinocerebellar ataxia type 1 (SCA1); and SCA2, 3, 6, and 7. In addition, three congenital fragile X syndromes, each associated with hypermethylation and unstable trinucleotide repeats, have been identified: FRAXA (CGG); FRAXE (GCC), and FRAXF (GCC). For each of the *FRA* genes, expression is extinguished by expansion and methylation.

<sup>2</sup>Although individuals with repeat length in the “premutation” size range are phenotypically normal, the corresponding chromosomes are very likely to expand to the “disease-length” category in the next meiosis.

<sup>3</sup>CGG, CTG, and CAG expansions are transcribed into the noncoding region of the mRNAs, whereas the CAG expansions associated with neurodegenerative disorders are translated into glutamine tracts.

(Adapted from Warren 1996.)

Strikingly, many other hereditary diseases of the nervous system involve similar expansions in trinucleotide repeats within the coding region of the gene responsible for the disease. These diseases include Friedreich's ataxia type 1, spinocerebellar ataxia, and certain spinal and bulbar muscular dystrophies (Table 3-1; Figure 3-13). By contrast, fragile X mental retardation is an X-linked recessive disease that involves a trinucleotide repeat in the control region near the coding region of the gene, leading to the inactivation of the *FMR* (fragile X mental retardation) gene. As in Huntington disease, progressive death of specific subpopulations of neurons or muscle cells occurs in many of these diseases.

### Most Complex Behavioral Traits in Humans Are Multigenic

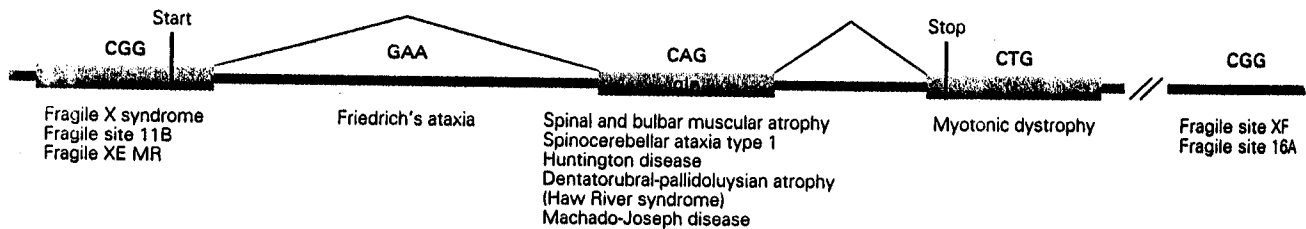
So far we have considered examples of the effects of single genes on behavior. Classic genetic analysis focuses on Mendelian traits, which, as we have seen, are

normally determined by allelic variation within a single gene. However, most behavioral traits as well as most common genetic disorders are multigenic; they are determined by several genes interacting with environmental factors.<sup>4</sup>

In contrast to single-locus Mendelian traits, multigenic traits do not have a simple recognizable pattern of inheritance (autosomal dominant, recessive, or X-linked), and thus the relative contributions of several genes to one trait is difficult to analyze. Nevertheless, determining which genes contribute to complex human traits has profound implications for the care and treatment of human disease.

Most common multigenic diseases, such as diabetes, coronary artery disease, asthma, schizophrenia,

<sup>4</sup>The term *multigenic* includes both oligogenic and polygenic traits. An *oligogenic* trait or disorder is determined by a small number of genes, each contributing to the phenotype in a significant way. In contrast, a *polygenic* trait is the result of many genes, each with a small effect on the phenotype.



**Figure 3-13** This model of a gene containing three exons and two introns (intervening blue line) depicts the location and type of expanded triplets involved in certain neurological diseases.

CGG repeats are found within the 5' untranslated region of the first exons of the genes for fragile X syndrome, fragile XE mental retardation (MR), and fragile site 11B. CGG repeats are also found at two fragile sites, XF and 16A, which are not known to be in the vicinity of any genes and, like fragile site 11B, are not known to result in any disease phenotype. GAA

repeats are found within the first exon of the X25 gene for Friedrich's ataxia. CAG repeats occur at five loci responsible for neurological diseases. These repeats are coding regions and thus result in the lengthening of a normal polyglutamine tract in their respective gene products. The repeats for Haw River syndrome are at the same locus as those for dentatorubral-pallidolusian atrophy and similarly involve expansion of the same CAG repeat. A CTG repeat (CAG on the other strand) occurs in the 3' untranslated region of the final exon of the protein kinase gene for myotonic dystrophy. (Adapted from Warren 1996.)

and manic-depressive disorder, are thought to represent a variety of disorders both etiologically and genetically. Thus, different mutant alleles and environmental factors are thought to produce indistinguishable phenotypes. In a typical multigenic disease, such as diabetes, there are scores of different alleles (among 10–12 different loci; see below) distributed throughout the human population of the world that are capable of contributing to the disease. In any one family three or four of these mutant alleles are likely to give rise to the disease. In fact, it is possible that each of the alleles that contributes to a multigenic disease functions as a normal polymorphism when expressed by itself but gives rise to disease if expressed together with other alleles in a certain genetic background. Moreover, because mono-zygotic twins with identical genetic endowment are often discordant for multigenic traits, the role of nongenetic factors must be important.

Several techniques have facilitated the genome-wide search for multigenic disorders in humans. The most common genetic mapping strategy is *linkage analysis*, in which a gene's locus is determined by comparing the inheritance of the mutant gene with a precisely mapped polymorphic DNA marker in a family afflicted with the particular disease. A DNA marker is useful if it maps to a unique locus within the human genome and it identifies frequent polymorphic variations between individuals at this locus. Coinheritance of a particular DNA marker with a mutant phenotype (or disease state) suggests that the marker and the mutant gene are physically close together on the chromosome.

Until 1980 polymorphisms could only be detected by differences in the behavior of the protein, for example, by differences in enzyme activity or electrophoretic mo-

bility. In the early 1980s it was appreciated that the non-coding regions, which make up 90–95% of the DNA, are the sites of frequent DNA polymorphisms. Indeed, single base pair changes that give rise to variants are relatively frequent in the human genome, with rates perhaps as high as 1 in 500 base pairs, and most of these changes occur in noncoding regions. The method of restriction fragment length polymorphisms (see Box 3-4) is used to detect polymorphisms throughout the genome.

The coinheritance of a DNA marker and mutant gene can occur by chance, or it can occur because the two loci recombine infrequently during meiosis, a direct result of their physical proximity. The chance that any two unlinked loci—for example, loci from different chromosomes—will be inherited together is  $1/2$ , and the chance that they will be coinherited in  $n$  siblings is  $(1/2)^n$ . Thus, if two loci are coinherited in all eight affected siblings from a single family, the odds against this being a random event would be  $(1/2)^8 = 256:1$ . In practice this is a more complicated event, one that is better analyzed by computer programs that calculate the ratio of the odds for and against linkage, while considering various statistical issues, and generate a value known as the *lod* (log of the odds) score. (For practical purposes a lod score equal to or greater than 3 indicates that evidence for linkage between a gene marker is significant. This represents odds of 20:1 in favor of linkage between the two loci.)

A related method of identifying polymorphisms is the characterization of simple sequence repeats by the polymerase chain reaction (PCR). The construction of high-resolution human genetic maps composed of these markers and the application of semi-automated screening technologies have facilitated linkage analysis. A

### Box 3-5 Analysis of Multigenic Traits

Quantitative trait locus (QTL) analysis is a method for identifying the multiple genes that condition a single behavioral trait. QTL analysis requires at least two strains of a species, each of which has been inbred until all members of the group are genetically identical and have two uniform sets of chromo-

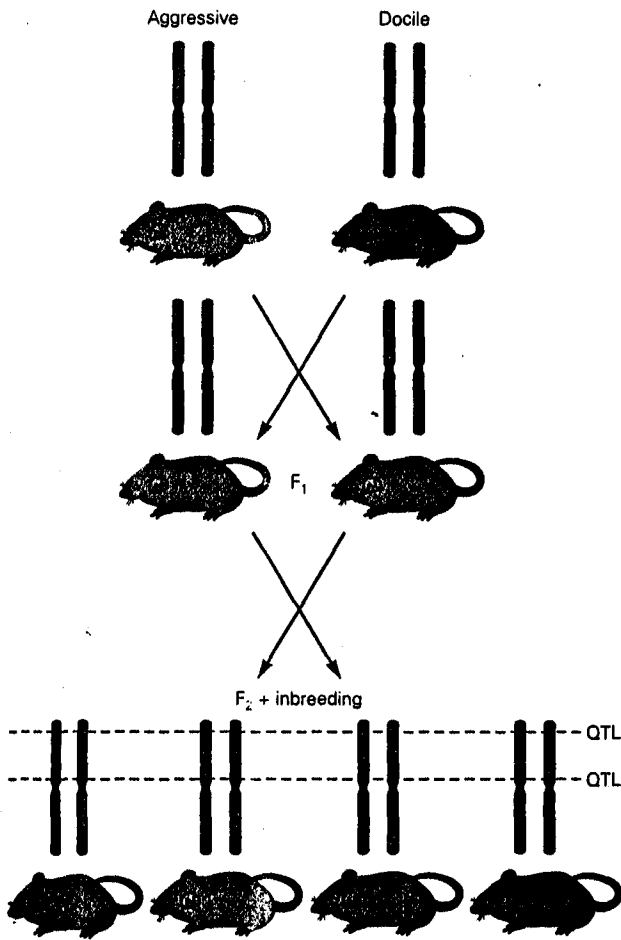


Figure 3-14.

somes. In the hypothetical example described here (Figure 3-14), two strains of mice have been selectively bred for aggressiveness (A) and docility (D).

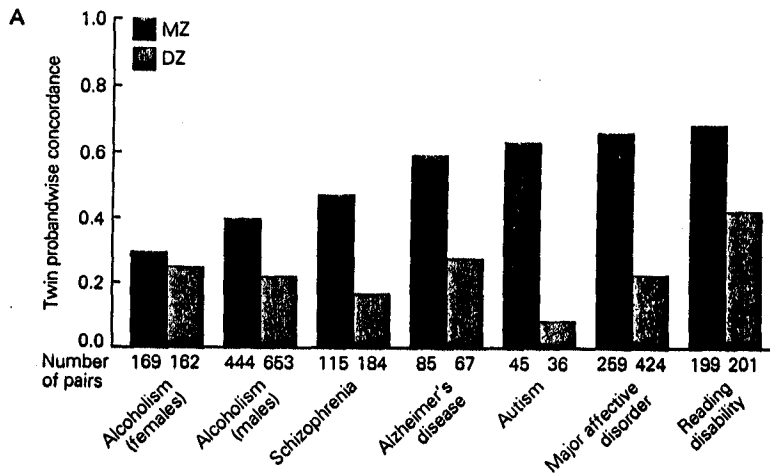
1. Aggressive A-type mice are bred with docile D-type mice, producing a first generation (F<sub>1</sub>) hybrid offspring in which every mouse has one set of chromosomes from each parent. In the F<sub>1</sub> generation the chromosomes in the cells that produce eggs and sperm exchange material. Segments of the mother's and father's DNA are recombined on individual chromosomes.
2. The F<sub>1</sub> generation is bred back to D-type mice, producing offspring with one recombinant set of chromosomes and one set that is pure D. In each offspring the recombinant chromosome will carry a unique mix of genes from both original strains.
3. Second-generation mice will show a range of aggressiveness because more than one gene determines aggressiveness and the mix of genes in the recombinant chromosome set varies. In Figure 3-14 the levels of aggressiveness in the second-generation mice are indicated by the different colors.
4. Sites in the genome that contain genes that contribute to aggression are identified by searching each mouse's DNA for genetic markers, landmarks scattered throughout the genome that are known to differ between the aggressive and docile strains. Each marker is examined to determine whether a mouse has inherited the A-type or D-type.
5. For each marker, the mice are sorted into those that have A-type DNA at that locus and those that have D-type DNA. The aggressiveness scores for the mice in the two groups are then compared. If the A-type group is significantly more aggressive than the D-type group, that marker represents a QTL that may contain a gene contributing to aggressiveness. Since each QTL interval contains many genes, additional methods must be used to find the one conditioning aggression.

(Modified from Barinaga 1994.)

gene that contributes to a multigenic trait is often called a *quantitative trait locus* (QTL) to indicate that it contributes to the genetic variance of a particular trait. QTL analysis is currently being used with mice and rats to track the genes that contribute to a number of behaviors (Box 3-5).

Linkage analysis is very sensitive to the model of transmission—dominant, recessive, X-linked, and oth-

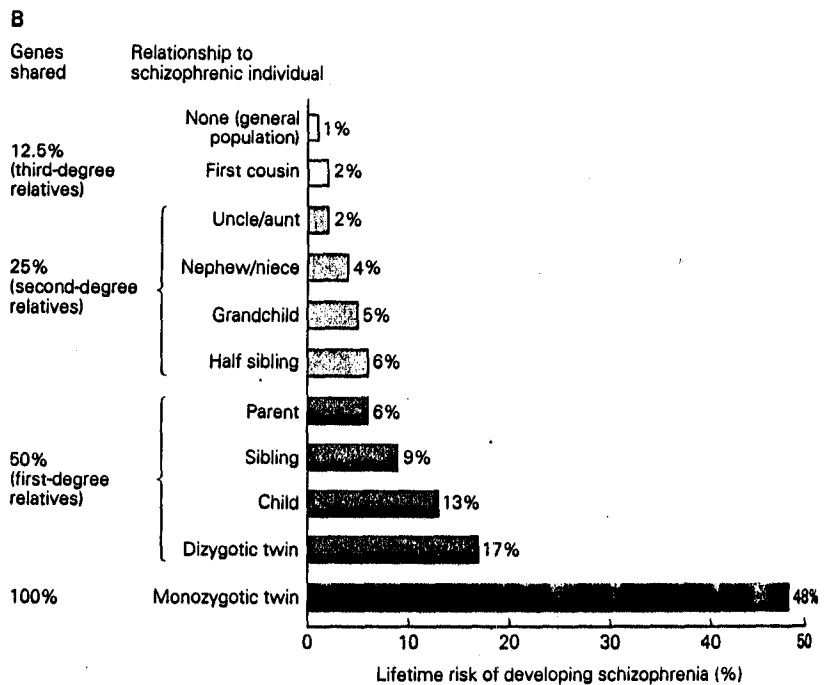
ers—and loses power when applied to multigenic traits where the mode of transmission is not known a priori. In the study of multigenic traits, therefore, researchers will often analyze the DNA marker data by linkage analysis (where genetic parameters must be specified prior to analysis) and by various nonparametric analyses that are much less dependent upon underlying genetic parameters. An example is sib-pair analysis where



**Figure 3-15.** Many complex human behavioral disorders have a genetic component.

**A.** Concordance for disease phenotypes among monozygotic twins (MZ) and dizygotic twins (DZ) for some behavioral disorders. The proband is the family member through which the family was initially discovered and explored. (Modified from Plomin et al. 1994.)

**B.** The risk of developing schizophrenia is a function of pedigree. (Modified from Gottesman 1991.)



one evaluates whether particular alleles (or chromosomal segments) are shared among affected siblings more often than would be predicted by chance alone. When the degree of allele-sharing reaches statistical significance, one concludes that the causal or predisposing mutation is contained within the shared region.

Family, twin, and adoption studies indicate not only that patients who suffer from the major psychiatric disorders have a genetic predisposition to those disorders but also that in the normal population at large components of character and general cognitive abilities have important genetic components. In the past it was generally assumed that these genetic contributions to charac-

ter and cognitive functioning decline over the course of one's lifetime because of the accumulation over the years of social and environmental experience. However, a study of the cognitive capabilities of 240 pairs of twins in the ninth decade of their life showed that genes continue to account for 50% of the variance in later life, much as they do earlier in life. Thus, while environmental factors are important, genes clearly contribute to a variety of normal higher mental functions.

Similarly, bipolar affective disorder (manic-depressive illness) frequently occurs in both siblings if they are monozygotic twins, but it occurs less frequently in both siblings if they are dizygotic twins. The heritability of

bipolar affective disorder, as well as that of schizophrenia, has been estimated to be about 50-60% (Figure 3-15). Thus, factors other than genes must play a critical role in determining the onset of disease in these multifactorial disorders.

Like other complex traits, schizophrenia and depression are most likely multigenic and multifactorial. It will be important to distinguish between various models of transmission. According to one (monogenic) model, many genes in the population can contribute to schizophrenia but each gene is rare and has a strong effect. Genetic linkage studies now indicate that such a monogenic model is likely to account for only a small fraction of schizophrenia patients. A second (oligogenic) model assumes that a small number of genes interact together to create a threshold of vulnerability for the disorder. Yet another (polygenic) model assumes that these disorders result from the cumulative effect of many genes, each with a minute effect. Several genetic forms of epilepsy most likely fit the monogenic model, whereas the major psychotic illnesses are thought to fit the oligogenic model. There may, however, be a subpopulation of people with major mental illness who suffer from the consequence of a powerful gene.

Schizophrenia and bipolar affective disorder were among the first multigenic traits to be analyzed by genetic linkage analysis. In fact, many of the early lessons learned from multigenic gene mapping came from mistakes made in these pioneering studies. Segregation analysis and genetic modeling studies indicate that both schizophrenia and bipolar disorder result from the effects of a small number of mutant genes. Thus, although mutations in a set of 10 or more genes may contribute to schizophrenia on a population basis (due to genetic heterogeneity), the combined effects of even a subset of these mutants would presumably be sufficient to place an individual at high risk for the disorder.

Furthermore, we know from twin studies that environmental and genetic factors together determine the overall likelihood of manifesting these disorders. According to this multifactorial model, a single mutation would produce a relatively small contribution to the overall predisposition to illness in the population and thus would be difficult to detect by genetic-linkage strategies. In any individual, however, one gene could actually be a quite strong contributor. For this reason, current psychiatric genetic studies usually involve international consortia cooperating in the systematic ascertainment and diagnosis of very large clinical samples, which lend sufficient power for the detection of small genetic contributions to illness. We shall see in Chapter 60 that the genotyping of several pedigrees has provided a possible genetic locus for susceptibility to schizophrenia.

## An Overall View

Most aspects of behavior are under genetic control. Evidence for this can be seen in the striking biological similarities of human twins and in our ability to select and breed domestic and laboratory animals for particular behavioral traits. Such breeding experiments generally indicate that behavioral traits are multigenic in origin. Only in rare instances has the source of natural variation been traceable to a single predominant genetic factor, as in the development of certain forms of obesity in mice.

Now, however, we are entering a new era in which it will be much easier to trace genes that control behavior. The availability of the complete genome for an organism will facilitate our understanding of how genes control genetic pathways important for cellular function, and this advance will allow much more effective and meaningful correlations with behavior. Several genomes are already completed: those of *Escherichia coli* and several other prokaryotic micro-organisms (5,000 genes, about 5 megabase (Mb) pairs), that of the yeast *Saccharomyces cerevisiae* (6,000 genes, 12 Mb), and that of the worm *Caenorhabditis elegans* (20,000 genes, 97 Mb). The human genome—all 80,000 genes—is likely to be completed by the year 2003, and work on the genomes of *Drosophila* and mouse are well underway. From the several genomes that have been completed we have already learned a number of surprising facts.

First, the human genome seems to have undergone two major replications from the primitive genome of single-celled organisms.

Second, fully 40% of the genes in yeast and *C. elegans* are novel; their function is completely unknown.

Third, from *C. elegans* we have learned that genes fall into two large classes that perform different functions and have different positions on the chromosomes. One set of 5,000 genes performs the core or housekeeping functions of the cell, the genes encode the proteins for intermediary metabolism for the metabolism of DNA, RNA and protein, for cytoskeletal structures, transport and secretion. The housekeeping genes are highly conserved, in both number and structure, and their ancestors have been found in yeast. Most likely they occur in comparable number in all organisms. In *C. elegans* these core function genes are clustered together in the central region of the chromosomes where they appear to be protected from evolutionary change.

The second set of about 15,000 genes are more specialized, and newer from an evolutionary perspective; they are not found in yeast. These specialized genes are mostly concerned with intercellular signaling, transcription, and other forms of regulatory control unique to multicellular organisms. These newer genes are posi-

tioned at the two ends of the chromosomes, where they appear to be more susceptible to evolutionary pressures. They include genes for 400 protein kinases, 480 zinc finger proteins that appear to be transcription factors, and 790 membrane-spanning receptors. Genes have been identified in *C. elegans* for most classes of human transcription factors and signaling proteins. In fact, many genes in *C. elegans* are similar to human genes involved in disease. Indeed, 70% of human proteins so far identified can be related to orthologs—similar proteins with a presumed common ancestor—in *C. elegans*.

Finally, simple perturbations of a yeast cell, such as the action of a mating factor, affects not a few but a large number of genes. Thus, in the future the perspective of genetic analysis will change from examining how single genes and proteins work to examining how many genes and proteins interact to produce a patterned response.

It is expected that the complete human genome, and the genomes of still other key organisms, will be to biology what the periodic table of elements has been to chemistry. For any species the genome will define all the genetic elements on which life's processes depend. The ability to analyze entire genomes promises to provide us with new insights that should dramatically change our ability to analyze behavioral processes, thereby altering dramatically the theory and practice of all areas of medicine, including neurology and psychiatry.

For example, what we already know about the human genome has brought molecular geneticists to the brink of identifying the combinations of genes contributing to certain multigenic disorders. The wealth of genetic information derived from such genetic linkage studies has enormous practical benefits. Researchers recently identified 10 to 12 different genes that predispose individuals to insulin-dependent diabetes mellitus. In addition, a variation in the number of repeating sequences within the gene encoding the dopamine D4 receptor is thought to make an important contribution to the overall genetic variance that characterizes novelty-seeking behavior. In the future the detection of genes that produce only a small effect on phenotype is likely to have a major impact on the study of behavioral disorders.

These findings raise fascinating issues about natural genetic variations in humans that we should be able to confront soon. To what degree do genetically transmitted differences in behavioral traits reflect quantitative variation in the expression of benign alleles and therefore natural variations of a *normal* behavior, as opposed to mutations of the same gene that produce a disease state? To what degree do the genetic contributions to natural variations in behavior reflect variations in the level of expression of the same protein? The answers to

such questions will be essential for developing rational therapeutic strategies for treating psychiatric disorders.

Variations in genes—in DNA sequences—represent the basic material for evolutionary change. These variations also form the basis for individual differences in risk for the many genetically complex diseases that confront neurology and psychiatry.

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