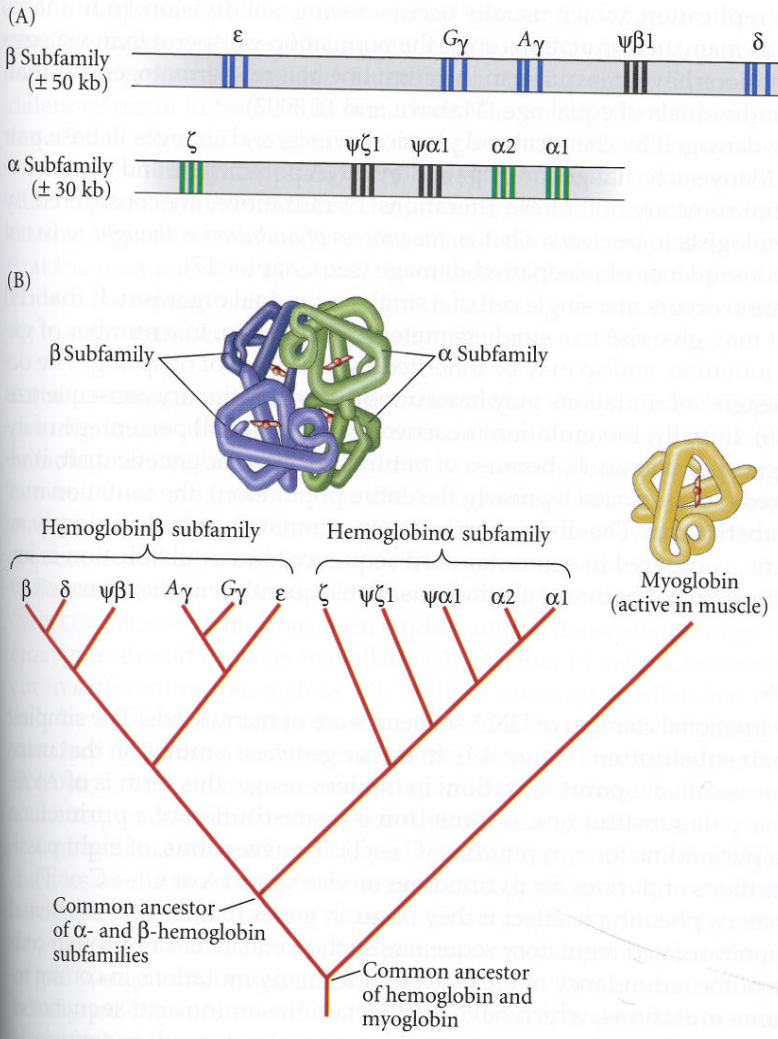


Figure 8.3 The human hemoglobin gene family has two subfamilies, α (green) and β (blue), located on different chromosomes. (A) Each functional gene is indicated by three lines, representing its three exons. Pseudogenes are denoted by ψ. (B) A gene tree for the hemoglobin gene family, with myoglobin as the outgroup. Myoglobin consists of a single protein unit. The hemoglobin protein (which carries oxygen in red blood cells) consists of four subunits, two each from the α and β subfamilies. (A after Lewin 1985; B after Hartwell et al. 2000.)

Gene Mutations

The word **mutation** refers both to the process of alteration of a gene or chromosome and to its product, the altered state of a gene or chromosome. It is usually clear from the context which is meant.

Before the development of molecular genetics, a mutation was identified by its effect on a phenotypic character. That is, a mutation was a newly arisen change in morphology, survival, behavior, or some other property that was inherited and could be mapped (at least in principle) to a specific locus on a chromosome. In practice, many mutations are still discovered, characterized, and named by their phenotypic effects. Thus we will frequently use the term "mutation" to refer to an alteration of a gene from one form, or **allele**, to another, the alleles being distinguished by phenotypic effects. In a molecular context, however, a gene mutation is an alteration of a DNA sequence, independent of whether or not it has any phenotypic effect. A particular DNA sequence that differs by one or more mutations from homologous sequences is called a **haplotype**. We will often refer to **genetic markers**, which are detectable mutations that geneticists use to recognize specific regions of chromosomes or genes.

Mutations have evolutionary consequences only if they are transmitted to succeeding generations. Mutations that occur in somatic cells may be inherited in certain animals and plants in which the reproductive structures arise from somatic meristems, but in those animals in which the germ line is segregated from the soma early in development, a mutation is inherited only if it occurs in a germ line cell. Mutations are thought to occur

mostly during DNA replication, which usually occurs during cell division. In hu
more than five times as many new mutations enter the population via sperm than v
because more cell divisions have transpired in the germ line before spermatogenes
before oogenesis in individuals of equal age (Makova and Li 2002).

DNA is frequently damaged by chemical and physical events, and changes in ba
sequence can result. Many such changes are repaired by DNA polymerase and by '
reading" enzymes, but some are not. These alterations, or mutations, are conside
most evolutionary biologists to be *errors*. That is, *the process of mutation is thought t*
an adaptation, but a consequence of unrepaired damage (see Chapter 17).

A particular mutation occurs in a single cell of a single individual organism. If t
is in the germ line, it may give rise to a single gamete, or quite often, to a numbe
metes that carry the mutation, and so may be inherited by a number of offspring.
currence of such "clusters" of mutations may have important evolutionary conseq
(Woodruff et al. 1996). Initially, the mutation is carried by a very small percentag
dividuals in the species population. If, because of natural selection or genetic dri
timately becomes fixed (i.e., is carried by nearly the entire population), the mutati
be referred to as a **substitution**. The distinction between a mutation, which is sim
altered form of a gene, compared to some standard sequence, and a substitutio
portant. Most mutations do not become substitutions. Consequently, *mutation is no*
alent to evolution.

Kinds of mutations

POINT MUTATIONS. Mutational changes of DNA sequences are of many kinds. The s
mutation is a **base pair substitution** (Figure 8.4). In classic genetics, a mutation tha
to a single gene locus is called a **point mutation**; in modern usage, this term is o
stricted to single base pair substitutions. A **transition** is a substitution of a puri
purine (A ↔ G) or a pyrimidine for a pyrimidine (C ↔ T). **Transversions**, of eigh
ble kinds, are substitutions of purines for pyrimidines or vice versa (A or G ↔ C o

Mutations may have a phenotypic effect if they occur in genes that encode rib
and transfer RNA, nontranslated regulatory sequences such as enhancers, or prote
ing regions. Because of the redundancy of the genetic code, many mutations in co
gions are **synonymous mutations**, which have no effect on the amino acid sequ
the polypeptide or protein. **Nonsynonymous mutations**, in contrast, result in ami
substitutions; they may have little or no effect on the functional properties of the p
tide or protein, and thus no effect on the phenotype, or they may have substantia
quences. For example, a change from the (RNA) triplet GAA to GUA causes the
acid valine to be incorporated instead of glutamic acid. This is the mutational ev
in humans caused the abnormal β-chain in sickle-cell hemoglobin, which has ma
notypic consequences and is usually lethal in homozygotes.

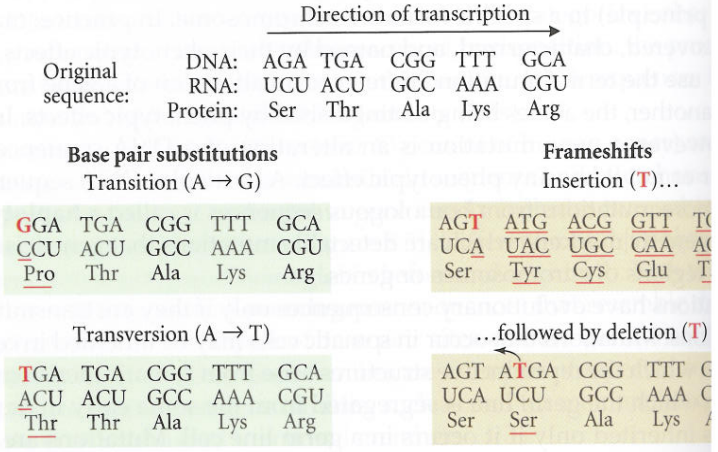


Figure 8.4 Examples of point mutations and their consequences for messenger RNA and amino acid sequences. (Only the transcribed, "sense" strand of the DNA is shown.) The boxes at the left show two kinds of base pair substitutions: a transition and a transversion at the first base position. The boxes at the right show two kinds of frameshift mutations.

If a single base pair becomes inserted into or deleted from a DNA sequence, the triplet reading frame is shifted by one nucleotide, so that downstream triplets are read as different codons and translated into different amino acids (Figure 8.4). Such insertions or deletions result in **frameshift mutations**. The greatly altered gene product is usually nonfunctional, although exceptions to this generalization are known.

A somewhat similar phenomenon is REPLICATION SLIPPAGE, which alters the number of short repeats in microsatellites. The growing (3′) end of a DNA strand that is being formed during replication may become dissociated from the template strand and form a loop, so that the next repeat to be copied from the template is one that had already been copied. Thus extra repeats will be formed in the growing strand. Microsatellite "alleles" that differ in copy number arise by replication slippage at a high rate.

SEQUENCE CHANGES ARISING FROM RECOMBINATION. Recombination typically is based on precise alignment of the DNA sequences on homologous chromosomes in meiosis. When homologous DNA sequences differ at two or more base pairs, **intragenic recombination** between them can generate new DNA sequences, just as crossing over between genes generates new gene combinations. DNA sequencing has revealed many examples of variant sequences that apparently arose by intragenic recombination.

Recombination appears to be the cause of a peculiar mutational phenomenon called GENE CONVERSION, which has been studied most extensively in fungi. The gametes of a heterozygote should carry its two alleles (A_1, A_2) in a 1:1 ratio. Occasionally, though, they occur in different ratios, such as 1:3. In these cases, an A_1 allele has been replaced specifically by an A_2 allele, rather than by any of the many other alleles to which it might have mutated: it seems to have been converted into A_2. This seems to occur when a damaged DNA strand of one chromosome is repaired by enzymes that insert bases complementary to the sequence on the undamaged homologous chromosome.

Unequal crossing over (unequal exchange) can occur between two homologous sequences or chromosomes that are not perfectly aligned. Recombination then results in a TANDEM DUPLICATION on one recombination product and a deletion on the other (Figure 8.5). The length of the affected region may range from a single base pair to a large block of loci (a segmental duplication), depending on the amount of displacement of the two misaligned chromosomes. Most unequal crossing over occurs between sequences that already include tandem repeats (e.g., ABBC) because the duplicate regions can pair out of register:

$$\begin{pmatrix}\text{ABBC...}\\ \text{...ABBC}\end{pmatrix}$$

This pairing generates further duplications (ABBBC). Unequal crossing over is one of the processes that have generated the extremely high number of copies of nonfunctional sequences that constitute much of the DNA in most eukaryotes. It has given rise to many gene families, and it has been extremely important in the evolution of greater numbers of functional genes and of total DNA (see Chapter 19).

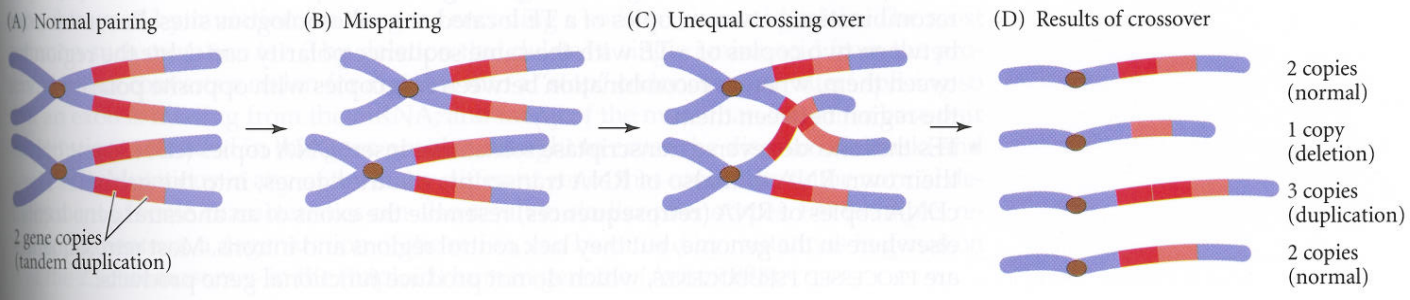


Figure 8.5 Unequal crossing over occurs most commonly when two repeated genes or sequences mispair with their homologues. Crossing over then yields a deletion of one of the sequences on one chromatid, and a duplication of the sequences (and hence three copies of that sequence) on the other chromatid. The duplicated sequence may encode part of a gene, or it may encode one or more complete genes. (After Hartl and Jones 2001.)

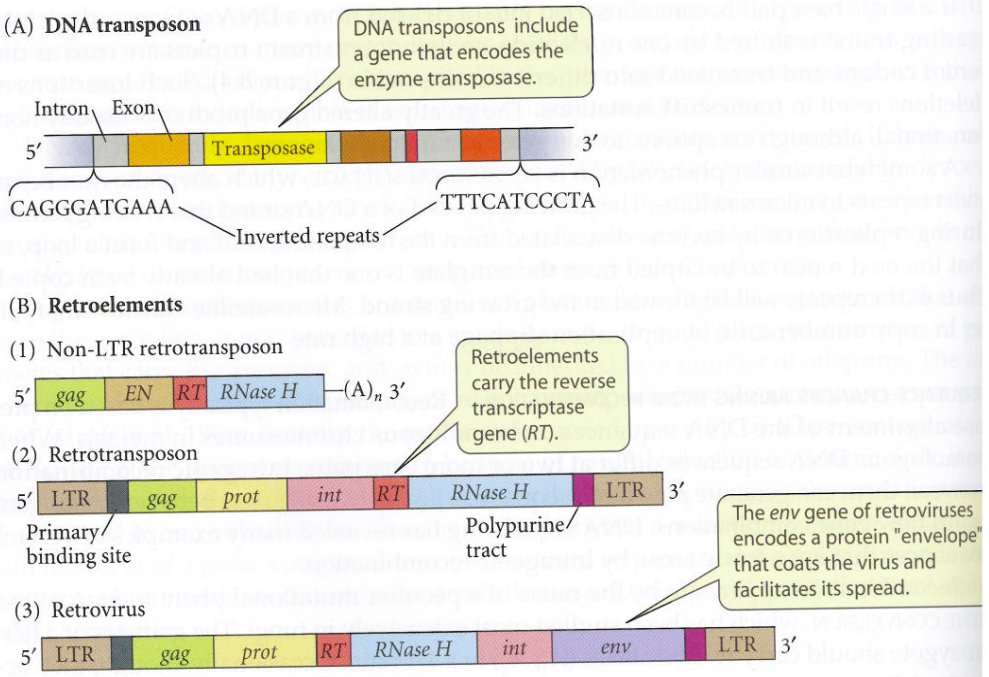


Figure 8.6 Some different kinds of transposable elements. (A) DNA transposable element are flanked by inverted repeats; these elements encode several genes in addition to a gene that encodes the enzyme (transposase) required for transposition. (B) Retroelements include a gene (*RT*) that encodes reverse transcriptase, which transcribes and RNA copy into DNA, which is then inserted in the genome. Three kinds of retroelements are shown. (1) Non-LTR retrotransposons, which include LINEs, lack long terminal repeats (LTRs). (2) Retrotransposons, such as *copia* in *Drosophila*, are similar but have LTRs. (3) Retroviruses, the most complex retroelements, include the gene (*env*) that encodes a protein envelope.

CHANGES CAUSED BY TRANSPOSABLE ELEMENTS. Most transposable elements produce copie that can move to any of many places in the genome, and sometimes they carry with them other genes near which they had been located. These DNA sequences include genes tha encode enzymes that accomplish the transposition (movement). The several kinds of TE (Figure 8.6) include INSERTION SEQUENCES, which encode only enzymes that cause trans position, TRANSPOSONS, which encode other functional genes as well, and RETROELEMENTS which carry a gene for the enzyme reverse transcriptase. Retroelements are first tran scribed into RNA, which then is reverse-transcribed into a DNA copy (cDNA) that is in serted into the genome. Some retroelements are retroviruses (including the HIV virus tha causes AIDS) whose RNA copies can cross cell boundaries. Retrotransposons, which in clude some LINE elements, are retroelements that act similarly, except that they do no cross cell boundaries and are copied only by cell division in the host.

Transposable elements often become excised from a site into which they had previously been inserted, but leave behind sequence fragments that tell of their former presence From such cases and from more direct evidence, transposable elements are known to hav many effects on genomes (Kazazian 2004; Bennetzen 2000):

- When inserted into a coding region, they alter, and usually destroy, the function of th protein, often by causing a frameshift or altering splicing patterns.
- When inserted into or near control regions, they can interfere with or alter gene ex pression (e.g., the timing or amount of transcription).
- They are known to increase mutation rates of host genes.
- They can cause rearrangements in the host genome, resulting from recombination be tween two copies of a TE located at different sites (Figure 8.7). Just as unequal crossin over between members of a gene family can generate duplications and deletions, so ca recombination between copies of a TE located at nonhomologous sites. Recombinatio between two copies of a TE with the same sequence polarity can *delete* the region be tween them, whereas recombination between two copies with opposite polarity *invert* the region between them.
- TEs that encode reverse transcriptase sometimes insert DNA copies (cDNA) not only o their own RNA, but also of RNA transcripts of other genes, into the genome. These cDNA copies of RNA (**retrosequences**) resemble the exons of an ancestral gene located elsewhere in the genome, but they lack control regions and introns. Most retrosequences are PROCESSED PSEUDOGENES, which do not produce functional gene products.

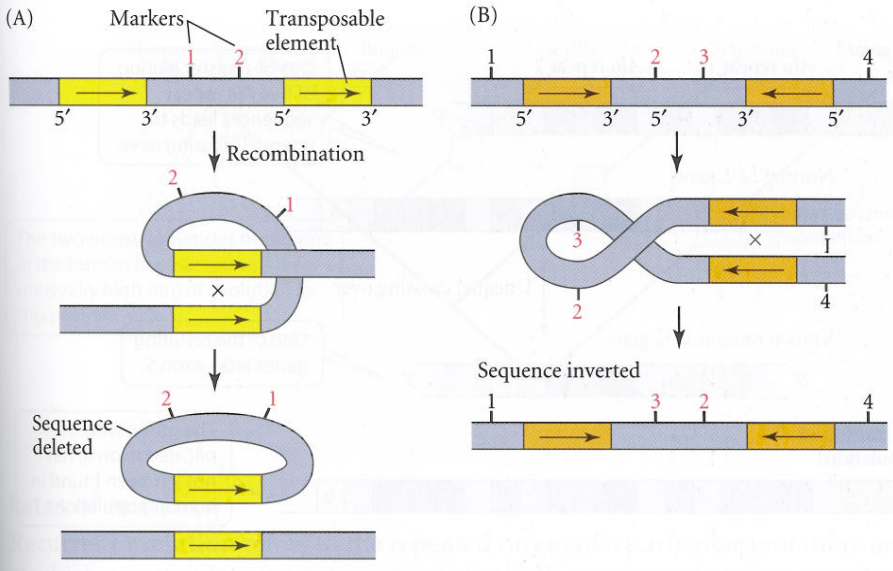


Figure 8.7 Recombination between copies of a transposable element can result in deletions and inversions. The boxes containing arrows represent transposable elements, with the polarity of base pair sequence indicated by the arrows. The numerals represent genetic markers. (A) Recombination (×) between two direct repeats (i.e., with the same polarity) excises one repeat and deletes the sequence between the two copies. (B) Recombination between two inverted repeats (with opposite polarity) inverts the sequence between them. (After Lewin 1985.)

- By transposition and unequal crossing over, TEs can increase in number, and so increase the size of the genome.

Most or all of these transposable element-induced effects have been observed within experimental populations of organisms such as maize (corn, *Zea mays*) and *Drosophila melanogaster*. The transposition rate of various retroelements ranges from about 10^{-5} to 10^{-3} per copy in inbred lines of *Drosophila*, resulting in an appreciable rate of mutation (Nuzhdin and Mackay 1994). All the kinds of changes engendered by transposable elements can be found by comparing genes and genomes of organisms of the same or different species. For instance, L1 retrotransposon insertions are associated with many disease-causing mutations in both mice and humans (Kazazian 2004), and a difference in flower color between two species of *Petunia* has been caused by the insertion and subsequent incomplete excision of a transposon that disabled a gene that controls anthocyanin pigment production (Quattrocchio et al. 1999).

Examples of mutations

Geneticists have learned an enormous amount about the nature and causes of mutations by studying model organisms such as *Drosophila* and *E. coli*. Moreover, many human mutations have been characterized because of their effects on health. Human mutations are usually rather rare variants that can be compared with normal forms of the gene; in some instances, newly arisen mutations have been found that are lacking in both of a patient's parents.

Single base pair substitutions are responsible for conditions such as sickle-cell anemia, described earlier, and for precocious puberty, in which a single amino acid change in the receptor for luteinizing hormone causes a boy to show signs of puberty when about 4 years old. Because many different alterations of a protein can diminish its function, the same phenotypic condition can be caused by many different mutations of a gene. For example, cystic fibrosis, a fatal condition afflicting one in 2500 live births in northern Europe, is caused by mutations in the gene encoding a sodium channel protein. The most common such mutation is a 3 bp deletion that deletes a single amino acid from the protein; another converts a codon for arginine into a "stop" codon; another alters splicing so that an exon is missing from the mRNA; and many of the more than 500 other base pair substitutions recorded in this gene are also thought to cause the disease (Zielenski and Tsui 1995). Mutations in any of the many different genes that contribute to the normal development of some characteristics can also result in similar phenotypes. For example, retinitis pigmentosa, a degeneration of the retina, can be caused by mutations in genes on 8 of the 23 chromosomes in the (haploid) human genome (Avise 1998).

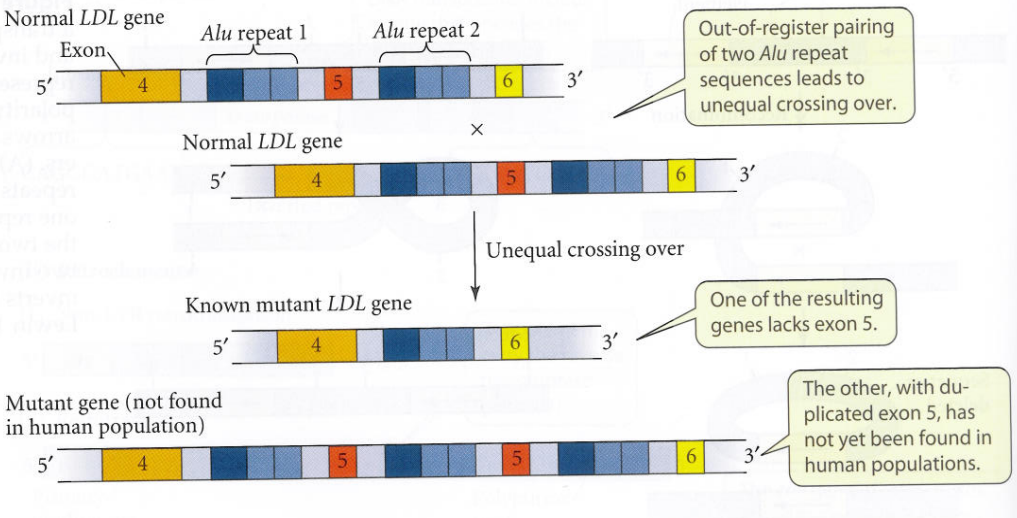


Figure 8.8 A mutated low-density lipoprotein (*LDL*) gene in humans lacks exon 5. It is believed to have arisen by unequal crossing over between two normal gene copies, due to out-of-register pairing between two of the repeated sequences (*Alu*, shown as blue boxes) in the introns. The numbered boxes are exons. (After Hobbs et al. 1986.)

Hemophilia can be caused by mutations in two different genes that encode blood-clotting proteins. In both genes, many different base pair substitutions, as well as small deletions and duplications that cause frameshifts, are known to cause the disease, and about 20 percent of cases of hemophilia-A are caused by an inversion of a long sequence within one of the genes (Green et al. 1995). Huntington disease, a fatal neurological disorder that strikes in midlife, is caused by an excessive number of repeats of the sequence CAG: the normal gene has 10 to 30 repeats, the mutant gene more than 75. Unequal crossing over between the two tandemly arranged genes for α-hemoglobin (see Figure 8.3) has given rise to variants with three tandem copies (duplication) and with one (deletion). The deletion of one of the loci causes α-thalassemia, a severe anemia. Another case of deletion, which results in high cholesterol levels, is the lack of exon 5 in a low-density lipoprotein gene. This deletion has been attributed to unequal crossing over, facilitated by a short, highly repeated sequence called *Alu* that is located in the introns of this gene and in many other sites in the genome (Figure 8.8).

These examples might make it seem as if mutations are nothing but bad news. While this is close to the truth—far more mutations are harmful than helpful—these mutations represent a biased sample. Many advantageous mutations have become incorporated into species' genomes (fixed) and thus represent the current **wild-type**, or normal, genes. For example, most genes that have arisen by reverse transcription from mRNA are nonfunctional pseudogenes, but at least one has been found that is a fully functional member of the human genome. Phosphoglycerate kinase is encoded by two genes. One, on the X chromosome, has a normal structure of 11 exons and 10 introns. The other, on an autosome, lacks introns, and clearly arose from the X-linked gene by reverse transcription. It is expressed only in the testes, a novel pattern of tissue expression that suggests that the gene plays a new functional role (see Li 1997).

When biologists seek those genes that have been involved in the evolution of a specific characteristic, they often use rare deleterious mutations of the kind described here as indicators of CANDIDATE GENES, those that may be among the genes they seek. For example, a rare mutation in the human *FOXP2* gene (*forkhead box 2*, which encodes a transcription factor) causes severe speech and language disorders. Two research groups, led by Jianzhi Zhang (Zhang et al. 2002) and Svante Pääbo (Enard et al. 2002), independently found that this gene has undergone two nonsynonymous (amino acid-changing) substitutions in the human lineage since the divergence of the human and chimpanzee lineages less than 7 Mya. This is a much higher rate of protein evolution than would be expected, considering that only one other such substitution has occurred between these species and the mouse, which diverged almost 90 Mya (Figure 8.9). Both research groups propose that these substitutions occurred in the human lineage less than 200,000 years ago and that they are among the important steps in the evolution of human language and speech.

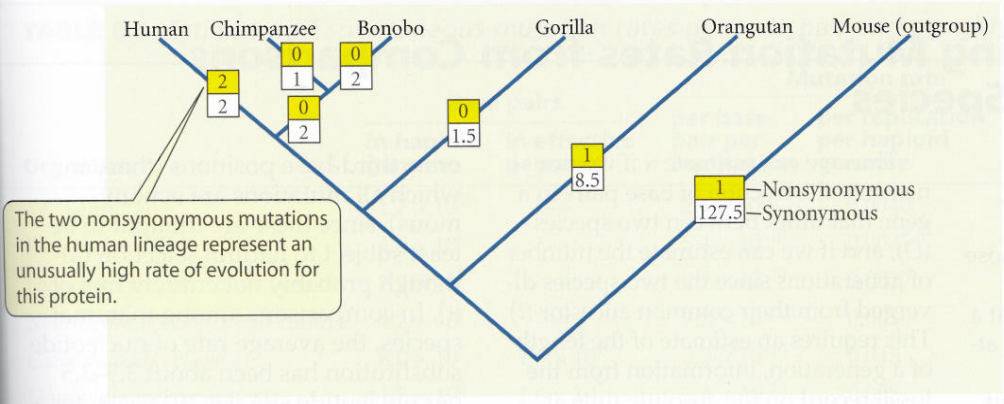


Figure 8.9 A phylogeny of the Hominoidea and its divergence from an outgroup, the mouse. Each box shows the number of nonsynonymous (yellow boxes) and synonymous (white boxes) substitutions in the *FOXP2* gene. The two nonsynonymous substitutions in the human lineage represent an unusually high rate of evolution of the FOXP2 protein, and may represent mutations that have been important in the evolution of language and speech. (After Zhang et al. 2002.)

Rates of mutation

Recurrent mutation refers to the repeated origin of a particular mutation, and the *rate* at which a particular mutation occurs is typically measured in terms of recurrent mutation: the number of independent origins per gene copy (e.g., per gamete) per generation or per unit time (e.g., per year). Mutation rates are estimates, not absolutes, and these estimates depend on the method used to detect mutations. In classical genetics, a mutation was detected by its phenotypic effects, such as white versus red eyes in *Drosophila*. Such a mutation, however, might be caused by the alteration of any of many sites within a locus; moreover, many base pair changes have no phenotypic effect. Thus phenotypically detected rates of mutation underestimate the rate at which all mutations occur at a locus. With modern molecular methods, mutated DNA sequences can be detected directly, so mutation rates can be expressed per base pair.

ESTIMATING MUTATION RATES. Rates of mutation are estimated in several ways (Drake et al. 1998). A relatively direct method is to count the number of mutations arising in a laboratory stock (which is usually initially homozygous), scoring mutations either by their phenotypic effects or by molecular methods. An indirect method (Box A) is based on the number of base pair differences between homologous genes in different species, relative to the number of generations that have elapsed since they diverged from their common ancestor. This method depends on the neutral theory of molecular evolution, which is described in Chapter 10.

Mutation rates vary among genes and even among regions within genes, but on average, as measured by phenotypic effects, a locus mutates at a rate of about 10^{-6} to 10^{-5} mutations per gamete per generation (Table 8.2). The average mutation rate per base pair, based mostly on the indirect method of comparing DNA sequences of different species, has been estimated at about 10^{-11} to 10^{-10} per replication in prokaryotes (see Table 8.3), or about 10^{-9} per sexual generation in eukaryotes. The mutation rate in the human genome has been estimated at about 4.8×10^{-9} per base pair per generation (Lynch et al. 1999).

Back mutation is mutation of a "mutant" allele back to the allele (usually the wild type) from which it arose. Back mutations are ordinarily detected by their phenotypic effects. They usually occur at a much lower rate than "forward" mutations (from wild type to mutant), presumably because many more substitutions can impair gene function than can restore it. At the molecular level, most phenotypically detected back mutations are not restorations of the original sequence, but instead result from a second amino

TABLE 8.2 *Spontaneous mutation rates of specific genes, detected by phenotypic effects*

Species and locus	Mutations per 100,000 cells or gametes
Escherichia coli	
Streptomycin resistance	0.00004
Resistance to T1 phage	0.003
Arginine independence	0.0004
Salmonella typhimurium	
Tryptophan independence	0.005
Neurospora crassa	
Adenine independence	0.0008–0.029
Drosophila melanogaster	
Yellow body	12
Brown eyes	3
Eyeless	6
Homo sapiens	
Retinoblastinoma	1.2–2.3
Achondroplasia	4.2–14.3
Huntington's chorea	0.5

Source: After Dobzhansky 1970.

BOX 8A Estimating Mutation Rates from Comparisons among Species

In Chapter 10, we will describe the *neutral theory of molecular evolution*. This theory describes the fate of purely neutral mutations—that is, those mutations that neither enhance nor lower fitness. One possible fate is that a mutation will become fixed—that is, attain a frequency of 1.0—entirely by chance. The probability that this event will occur equals u, the rate at which neutral mutations arise. In each generation, therefore, the probability is u that a mutation that occurred at some time in the past will become fixed. After the passage of t generations, the fraction of mutations that will have become fixed is therefore ut.

If two species diverged from a common ancestor t generations ago, the expected fraction of fixed mutations in both species is $D = 2ut$, since various mutations have become fixed in both lineages. If the mutations in question are base pair changes, a fraction $D = 2ut$ of the base pairs of a gene should differ between the species, assuming that all base pairs are equally likely to mutate. Thus the average mutation rate per base pair per generation is $u = D/2t$.

Thus we can estimate u if we can measure the fraction of base pairs in a gene that differ between two species (D), and if we can estimate the number of generations since the two species diverged from their common ancestor (t). This requires an estimate of the length of a generation, information from the fossil record on the absolute time at which the common ancestor existed, and an understanding of the phylogenetic relationships among the living and fossilized taxa.

In applying this method to DNA sequence data, it is necessary to assume that most base pair substitutions are neutral and to correct for the possibility that earlier substitutions at some sites in the gene have been replaced by later substitutions ("multiple hits"). Uncertainty about the time since divergence from the common ancestor is usually the greatest source of error in estimates obtained by this method.

The best estimates of mutation rates at the molecular level have been obtained from interspecific comparisons of pseudogenes, other nontranslated sequences, and fourfold-degenerate third-base positions (those in which all mutations are synonymous), since these are thought to be least subject to natural selection (although probably not entirely free of it). In comparisons among mammal species, the average rate of nucleotide substitution has been about 3.3–3.5 per nucleotide site per 10^9 years, for a mutation rate of $3.3–3.5 \times 10^{-9}$ per site per year (Li and Graur 1991). If the average generation time were 2 years during the history of the lineages studied, the average rate of mutation per site would be about 1.7×10^{-9} per generation. Comparison of human and chimpanzee sequences yielded an estimate of 1.3×10^{-9} per site per year, assuming divergence 7 Mya. If the average generation time in these lineages has been 15–20 years, then the mutation rate is about 2×10^{-8} per generation. The human diploid genome has 6×10^9 nucleotide pairs, so this implies at least 120 new mutations per genome per generation—an astonishingly high number (Crow 1993).

acid substitution, either in the same or a different protein, that restores the function that had been altered by the first substitution. Advantageous mutations arose and compensated for severely deleterious mutations within 200 generations in experimental populations of *E. coli* (Moore et al. 2000).

EVOLUTIONARY IMPLICATIONS OF MUTATION RATES. With such a low mutation rate per locus, it might seem that mutations occur so rarely that they cannot be important. However, summed over all genes, the input of variation by mutation is considerable. If the haploid human genome has 3.2×10^9 bp and the mutation rate is 4.8×10^{-9} per bp per generation, an average zygote will carry about 317 new mutations. If only 2.5 percent of the genome consists of functional, transcribed sequences, 7 of these new mutations will be expressed and will have the potential to affect phenotypic characters (Lynch et al. 1999). Other authors have estimated the number of new base pair changes per zygote in the functional part of the genome as 0.14 in *Drosophila*, 0.9 in mice, and 1.6 in humans (Table 8.3). So, in a population of 500,000 humans, at least 800,000 new mutations arise every generation. If even a tiny fraction of these mutations were advantageous, the amount of new "raw material" for adaptation would be substantial, especially over the course of thousands or millions of years.

Experiments on *Drosophila* have confirmed that the total mutation rate per gamete is quite high. For example, Terumi Mukai and colleagues (1972), in a heroically large experiment, counted more than 1.7 million flies in order to estimate the rate at which the

TABLE 8.3 *Estimates of spontaneous mutation rates per base pair and per genome*

Organism	Base pairs		Mutation rate			
	in haploid genome	in effective genome[a]	per base pair per replication	per replication per haploid genome	per replication per effective genome[a]	per sexual generation per effective genome[b]
T2, T4 phage	1.7×10^5	—	2.4×10^{-8}	0.0040	—	—
Escherichia coli	4.6×10^6	—	5.4×10^{-10}	0.0025	—	—
Saccharomyces cerevisiae (yeast)	1.2×10^7	—	2.2×10^{-10}	0.0027	—	—
Neurospora crassa (bread mold)	4.2×10^7	—	7.2×10^{-11}	0.0030	—	—
Caenorhabditis elegans	8.0×10^7	1.8×10^7	2.3×10^{-10}	0.018	0.004	0.036
Drosophila melanogaster	1.7×10^8	1.6×10^7	3.4×10^{-10}	0.058	0.005	0.14
Mouse	2.7×10^9	8.0×10^7	1.8×10^{-10}	0.49	0.014	0.9
Human	3.2×10^9	8.0×10^7	5.0×10^{-11}	0.16	0.004	1.6

Source: After Drake et al. 1998.
[a] The effective genome is the number of base pairs in functional sequences that could potentially undergo mutations that reduce fitness.
[b] Calculated for multicellular organisms in which multiple DNA replication events occur in development between zygote and gametogenesis.

chromosome 2 accumulates mutations that affect egg-to-adult survival (VIABILITY). They used crosses (see Figure 9.7) in which copies of the wild-type chromosome 2 were carried in a heterozygous condition so that deleterious recessive mutations could persist without being eliminated by natural selection. Every 10 generations, they performed crosses that made large numbers of these chromosomes homozygous and measured the proportion of those chromosomes that reduced viability. The mean viability declined, and the variation (variance) among chromosomes increased steadily (Figure 8.10). From the changes in the mean and variance, Mukai et al. calculated a mutation rate of about 0.15 per chromosome 2 per gamete. This is the sum, over all loci on the chromosome, of mutations that affect viability. Because chromosome 2 carries about a third of the *Drosophila* genome, the total mutation rate is about 0.50 per gamete. Thus almost every zygote carries at least one new mutation that reduces viability. Subsequent studies have indicated that the mutation rate for *Drosophila* is at least this high, and that it reduces viability by 1 to 2 percent per generation (Lynch et al. 1999). Indirect estimates indicate that humans likewise suffer about 1.6 new mutations per zygote that reduce survival or reproduction (Eyre-Walker and Keightley 1999).

Mutation rates vary among genes and chromosome regions, and they are also affected by environmental factors. MUTAGENS (mutation-causing agents) include ultraviolet light, X-rays, and a great array of chemicals, many of which are environmental pollutants. For example, mutation rates in birds and mice are elevated in industrial areas, and mice exposed to particulate air pollution in an urban-industrial site showed higher rates of mutation in repetitive elements than mice exposed only to filtered air at that site, or mice placed in a rural location (Figure 8.11).

Figure 8.12 shows that the variation in a typical phenotypic character is **polygenic**: it is based on several or many different genetic loci. It is very difficult to single out any of these loci to study the mutation rate per locus, but it is easy to estimate the **mutational variance** of the character—the increased variation in a population caused by new mutations in each generation. Studies of traits such as bristle number in *Drosophila* have shown that the mutational variance is high enough that an initially homozygous population would take only about 500 generations to achieve the level of genetic variation generally found in a natural population. The magnitude of the mutational variance varies somewhat among characters and species (Lynch 1988).

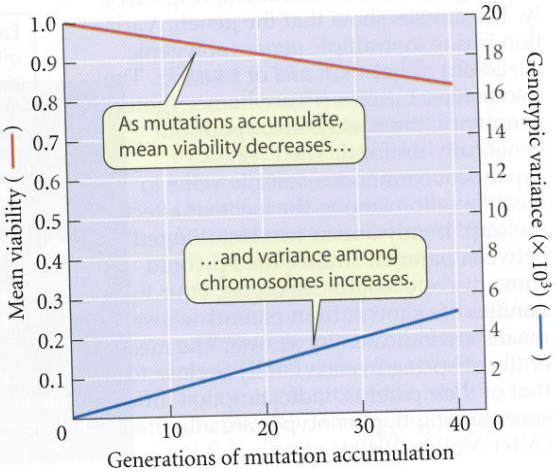


Figure 8.10 Effects of the accumulation of spontaneous mutations on the egg-to-adult survival of *Drosophila melanogaster*. The mean viability of flies made homozygous for chromosome 2 carrying new recessive mutations decreased, and the variation (variance) among those chromosomes increased. The rate of mutation was estimated from these data. (After Mukai et al. 1972.)

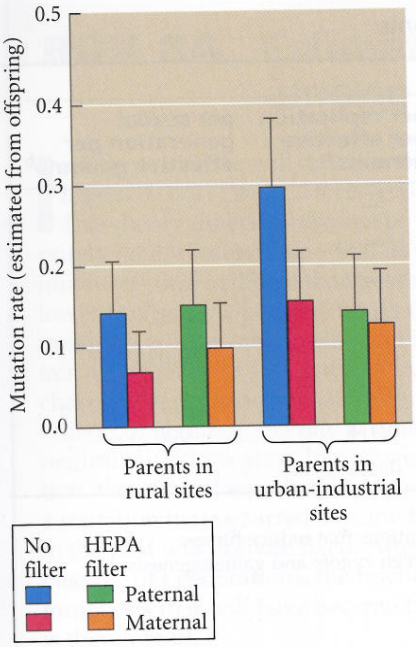


Figure 8.11 Mutation rates in mice, estimated from DNA sequences of two loci in their offspring. The mice were placed for 10 weeks in rural sites or in urban-industrial sites near steel mills and a major highway, where they were exposed either directly to the air or to air passed through a HEPA filter. Exposure to unfiltered urban-industrial air increased the mutation rate. (After Somers et al. 2004.)

In summary, although any given mutation is a rare event, the rate of origination of new genetic variation in the genome as a whole, and for individual polygenic characters, is appreciable. However, mutation alone does not cause a character to evolve from one state to another, because its rate is too low. Suppose that alleles A_1 and A_2 determine alternative phenotypes (e.g., red versus purple) of a haploid species, that half the individuals carry A_1, and that the rate of recurrent mutation from A_1 to A_2 is 10^{-5} per gene per generation. In one generation, the proportion of A_2 genes will increase from 0.5 to $[0.5 + (0.5)(10^{-5})] = 0.50000495$. At this rate, it will take about 70,000 generations before A_2 constitutes 75 percent of the population, and another 70,000 generations before it reaches 87 percent. This rate is so slow that, as we will see, factors other than recurrent mutation usually have a much stronger influence on allele frequencies, and thus are responsible for whatever evolutionary change occurs.

Phenotypic effects of mutations

A mutation may alter one or more phenotypic characters, such as size, coloration, or the amount or activity of an enzyme. Alterations in such features may affect survival and/or reproduction, the major components of FITNESS (see Chapter 11). It is often con-

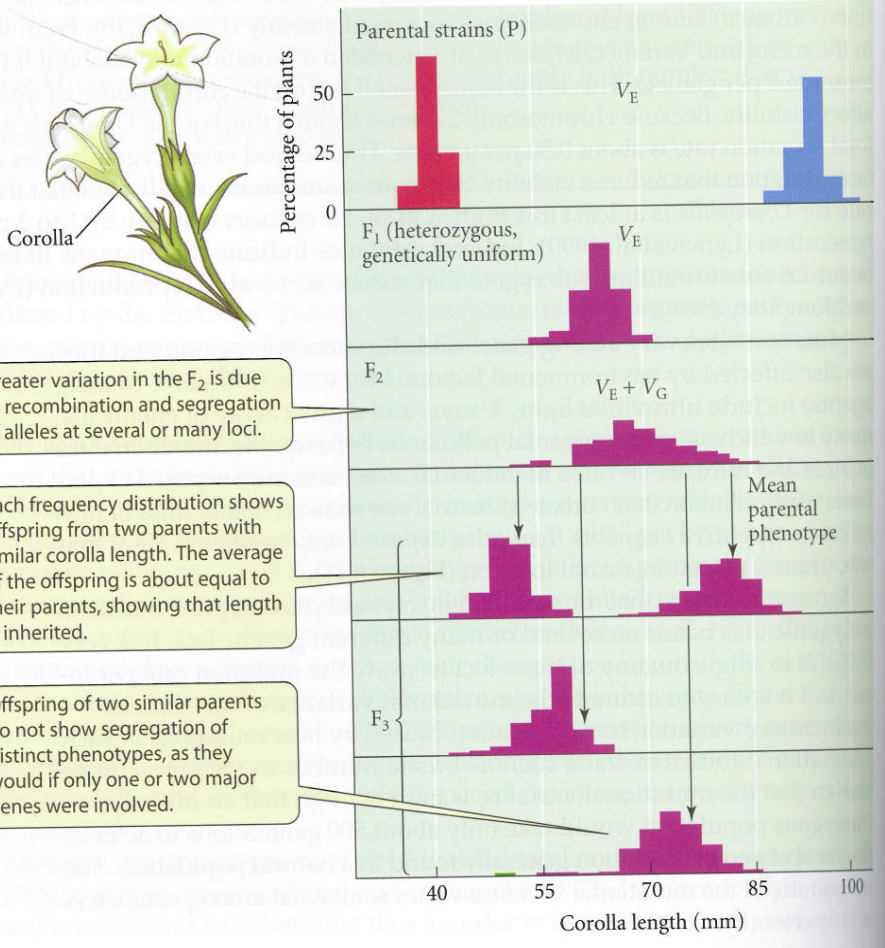


Figure 8.12 Inheritance of a continuously varying trait, length of the corolla (petals), in the tobacco plant *Nicotiana longiflora*. V_G and V_E represent variation due to genes and environment, respectively. The crosses show that the genetic variation is due to multiple genes (polygenic variation) rather than one or two loci. The two parental strains (P) are homozygous genotypes; the F_1 is heterozygous but genetically uniform. The F_2 shows expanded, continuous variation due to recombination among the loci that affect the trait. If only one or two loci differed between parental strains, the F_2 would show discrete length categories. Four F_3 families are shown, from parents whose means are indicated by arrows. The mean of the offspring in each family is close to that of their parents, indicating that differences among F_2 phenotypes are inherited. (After Mather 1949.)

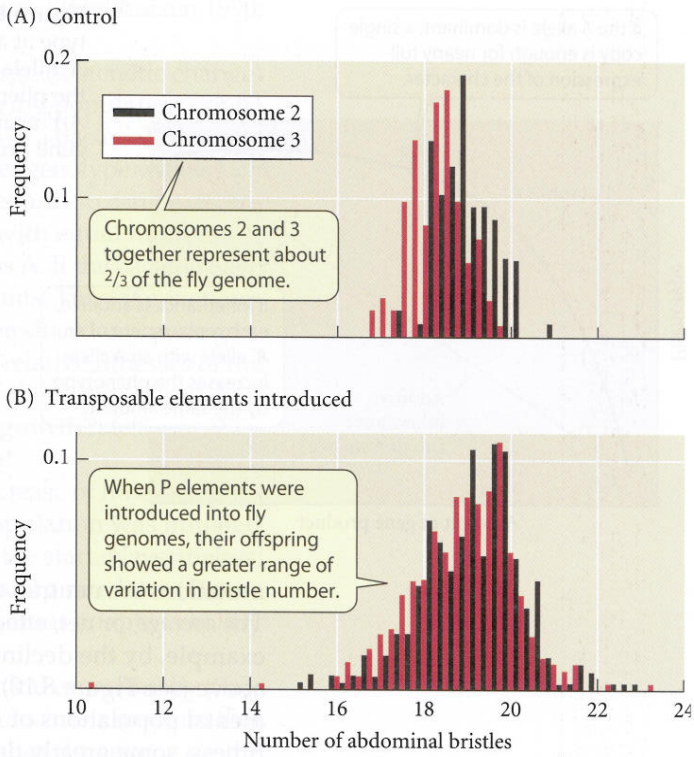


Figure 8.13 The frequency distribution of the number of abdominal bristles in (A) 392 homozygous control lines of *Drosophila melanogaster* and (B) 1094 homozygous experimental lines in which researchers used transposable elements (P elements) to induce mutations in chromosome 2 or chromosome 3. The mutations both increased and decreased bristle numbers compared with the control lines. (From Lyman et al. 1996.)

venient to distinguish between a mutation's effects on fitness and on other characters, even though they are connected.

The phenotypic effects of mutational changes in DNA sequence range from none to drastic. At one extreme, synonymous base pair changes are expected to have no evident phenotypic effect, and this is also apparently true of many amino acid substitutions, which seem not to affect protein function. The phenotypic effects of mutations that contribute to polygenic traits, such as bristle number in *Drosophila*, range from slight to substantial; in one study, mutations induced by insertion of transposable elements altered the number of abdominal bristles by about 0.9 bristle on average (Figure 8.13).

Among the most fascinating mutations are those in the "master control genes" that regulate the expression of other genes in developmental pathways. (We will discuss these genes in detail in Chapter 20.) The HOMEOTIC SELECTOR GENES, for example, determine the basic body plan of an organism, conferring a distinct identity on each segment of the developing body by producing DNA-binding proteins that regulate other genes that determine the features of each such segment. These genes derive their name from **homeotic mutations** in *Drosophila*, which redirect the development of one body segment into that of another. Mutations in the Antennapedia gene complex, for example, cause legs to develop in place of antennae (Figure 8.14). Another master control gene, *Pax6*, switches on about 2500 other genes required for eye development in mammals, insects, and many other animals (Gehring and Ikeo 1999). Mutations in this gene cause malformation or loss of eyes.

Dominance describes the effect of an allele on a phenotypic character when it is paired with another allele in the heterozygous condition. A fully dominant allele (say, A_1) produces nearly the same phenotype when heterozygous (A_1A_2) as when homozygous (A_1A_1), and its partner allele (A_2) in that instance is fully **recessive**. All degrees of INCOMPLETE DOMINANCE, measured by the degree to which the heterozygote resembles one or the other homozygote, may occur. Inheritance is said to be **additive** if the heterozygote's phenotype is precisely intermediate between those of the homozygotes. For example, A_1A_1,

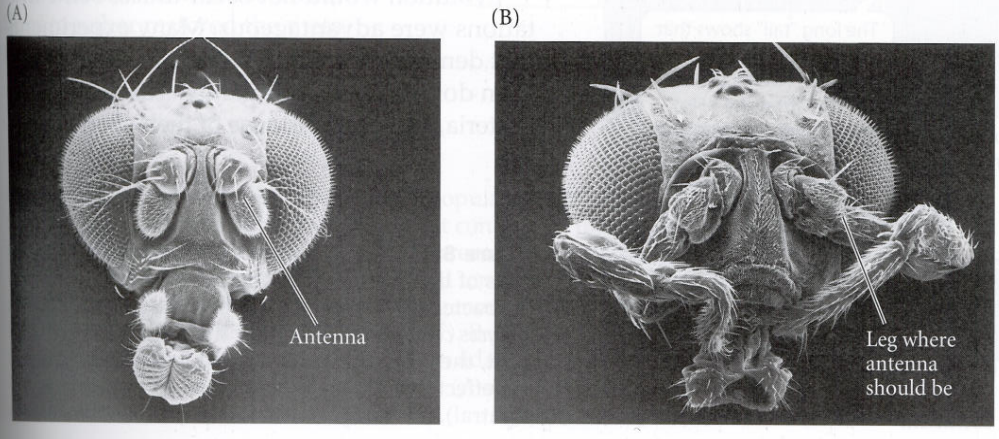


Figure 8.14 The drastic phenotypic effect of homeotic mutations that switch development from one pathway to another. (A) Frontal view of the head of a wild-type *Drosophila melanogaster*, showing normal antennae and mouthparts. (B) Head of a fly carrying the *Antennapedia* mutation, which converts antennae into legs. The *Antennapedia* gene is part of a large complex of Hox genes that confer identity on segments of the body (see Chapter 20). (Photographs courtesy of F. R. Turner.)

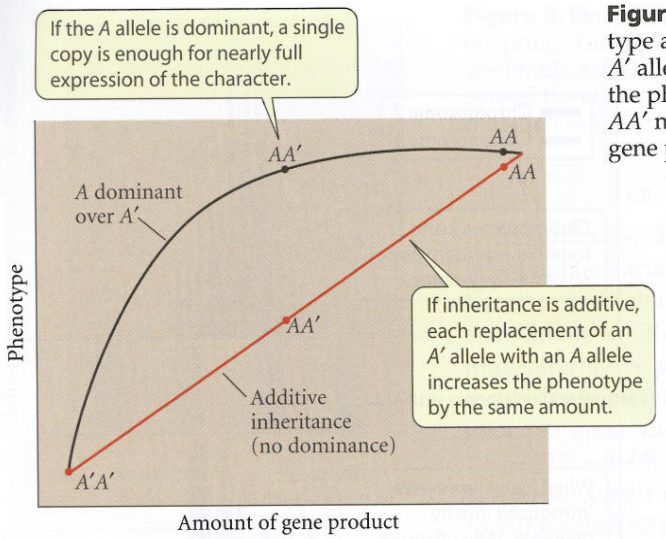


Figure 8.15 Two of the possible relationships between phenotype and genotype at a single locus with two alleles. If inheritance is additive, replacing each A' allele with an A allele steadily increases the amount of gene product, and the phenotype changes accordingly. If A is dominant over A', the phenotype of AA' nearly equals that of AA because the single dose of A produces enough gene product for full expression of the character.

A_1A_2, and A_2A_2 may have phenotypes 3, 2, and 1, respectively; the effects of replacing each A_2 with an A_1 simply add up. LOSS-OF-FUNCTION mutations, in which the activity of a gene product is reduced, are often at least partly recessive, while dominant mutations often have enhanced gene product activity (Figure 8.15).

Effects of mutations on fitness

The effects of new mutations on fitness may range from highly advantageous to highly disadvantageous. Undoubtedly, many mutations are neutral, or nearly so, having very slight effects on fitness (see Chapter 10). The *average*, or net, effect of those that do affect fitness is deleterious. This was shown, for example, by the decline in mean viability in Mukai's *Drosophila* experiment, described above (see Figure 8.10), and by the fitness effects of single mutations isolated in experimental populations of *E. coli* and yeast (Figure 8.16). A few mutations slightly enhanced fitness, some greatly decreased it, and the majority had small deleterious effects. Under some circumstances, slightly deleterious mutations may act as if they are nearly neutral and accumulate in populations. They may therefore have more harmful collective effects on a population than do strongly deleterious mutations, which are more rapidly expunged by natural selection.

The frequency distribution of mutational effects is not fixed, for the fitness consequences of many mutations depend on the population's environment and even on its existing genetic constitution. For example, the decline of fitness due to new mutations in some experimental *Drosophila* populations was more than 10 times greater if the flies were assayed under crowded, competitive conditions than under noncompetitive conditions (Shabalina et al. 1997).

Most mutations are **pleiotropic**—that is, they affect more than one character. For example, the *yellow* mutation in *Drosophila* affects not only body color, but also several components of male courtship behavior. In some cases, the basis of deleterious pleiotropic effects is understood; for example, some mutations that affect *Drosophila* bristle number also disrupt the development of the nervous system and reduce the viability of larvae, which do not have bristles (Mackay et al. 1992).

Evolution would not occur unless some mutations were advantageous. Many experiments that demonstrate advantageous mutations have been done with microorganisms such as phage, bacteria, and yeast because of their short genera-

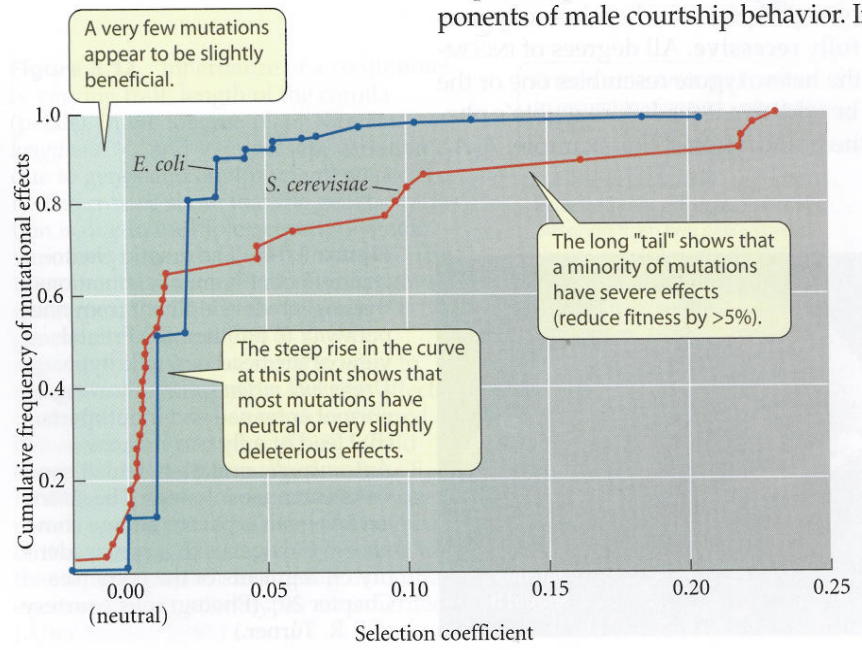


Figure 8.16 The cumulative frequency distributions of the effects of new mutations on fitness in the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae*. The higher the selection coefficient, the more the mutation reduces fitness. Beneficial effects are indicated by values to the left of 0.0 (neutral). (After Lynch et al. 1999.)

tion times and the ease with which huge populations can be cultured (Dykhuizen 1990; Elena and Lenski 2003).

Because bacteria can be frozen (during which time they undergo no genetic change) and later revived, samples taken at different times from an evolving population can be stored, and their fitness can later be directly compared. The fitness of a bacterial genotype is defined as its rate of increase in numbers relative to that of another genotype with which it competes in the same culture, but which bears a genetic marker and so can be distinguished from it. Suppose, for example, that a culture is begun with equal numbers of genotypes A and B, and that after 24 hours B is twice as abundant as A. If the bacteria have grown for x generations, each initial cell has produced 2^x descendants. Thus, if genotypes A and B have grown at the respective rates of 2^5 and 2^6 (i.e., B has produced one more generation per 24 hours), their relative numbers are 32:64, or 1:2. The relative fitnesses of the genotypes—that is, their relative growth rates—are measured by their rates of cell division per day: namely, 5:6, or 1.0:1.2. If the genotypes had the same growth rates—say, 2^5—both would increase in number, but their fitnesses would be equal.

Richard Lenski and colleagues used this method to trace the increase of fitness in populations of *E. coli* for an astonishing 20,000 generations. Each population was initiated with a single individual and was therefore genetically uniform at the start. Nevertheless, fitness increased substantially—rapidly at first, but at a decelerating rate later (Figure 8.17A). In a similar experiment (Bennett et al. 1992), *E. coli* populations adapted rapidly to several different temperatures (Figure 8.17B,C,D).

Bacteria can be screened for mutations that affect their biochemical capacities by placing them on a medium on which that bacterial strain cannot grow, such as a medium that lacks an essential amino acid or other nutrient. Whatever colonies do appear on the medium must have grown from the few cells in which mutations occurred that conferred a new biochemical ability. For example, Barry Hall (1982) studied a strain of *E. coli* that lacks the *lac*Z gene, which encodes β-galactosidase, the enzyme that enables *E. coli* to metabolize the sugar lactose as a source of carbon and energy. Hall screened populations for the ability to grow on lactose and recovered several mutations. A mutation in a different gene (*ebg*) altered an enzyme that normally performs another function so that it could break down lactose. Another mutation altered regulation of the *ebg* gene, and a third mutation altered the ebg enzyme so that it metabolized lactose into lactulose, which increased the cell's uptake of lactose from the environment. The three mutations together restored the metabolic capacities that had been lost

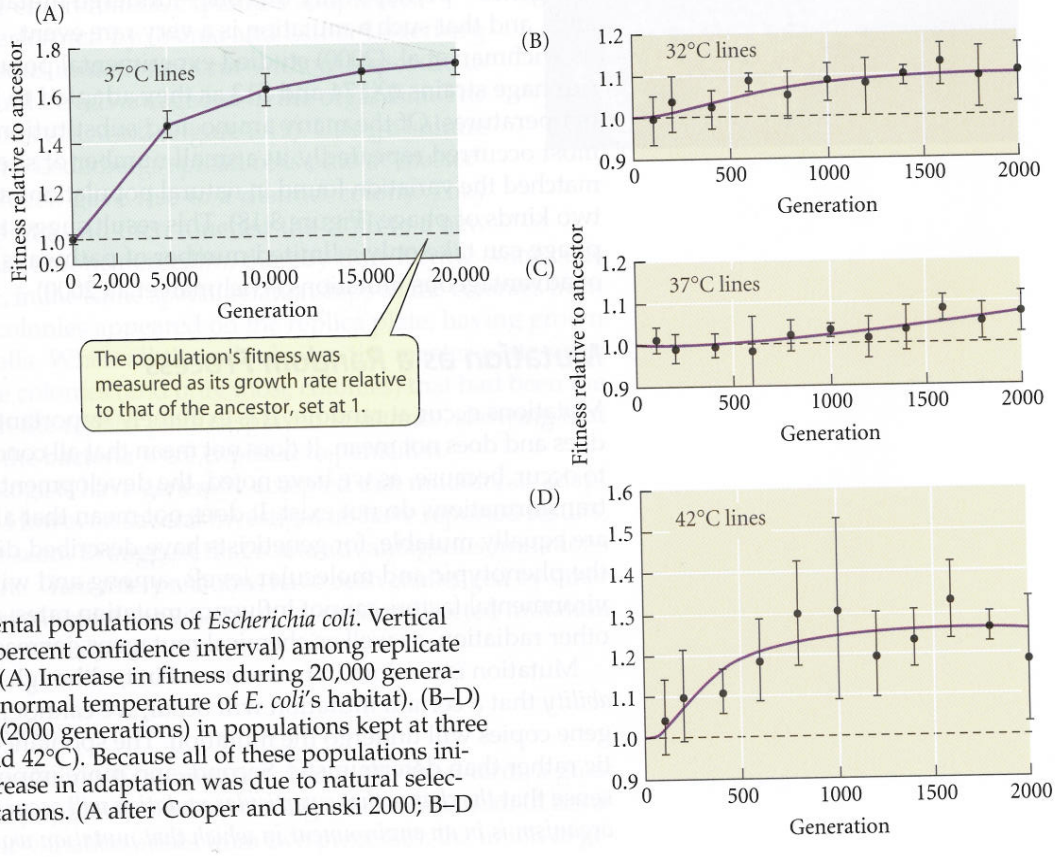


Figure 8.17 Adaptation in experimental populations of *Escherichia coli*. Vertical bars show a measure of variation (95 percent confidence interval) among replicate populations around the mean fitness. (A) Increase in fitness during 20,000 generations in populations kept at 37°C (the normal temperature of *E. coli*'s habitat). (B–D) Adaptation over a much shorter time (2000 generations) in populations kept at three different temperatures (32°C, 37°C, and 42°C). Because all of these populations initially lacked genetic variation, the increase in adaptation was due to natural selection acting on new advantageous mutations. (A after Cooper and Lenski 2000; B–D after Bennett et al. 1992.)

by the deletion of the original *lacZ* gene. Thus mutation and selection in concert can give rise to complex adaptations.

The limits of mutation

It cannot be stressed too strongly that even the most drastic mutations cause alterations of one or more *pre-existing* traits. Mutations with phenotypic effects alter developmental processes, but they cannot alter developmental foundations that do not exist. We may conceive of winged horses and angels, but no mutant horses or humans will ever sprout wings from their shoulders, for the developmental foundations for such wings are lacking.

The direction of evolution may be constrained if some conceivable mutations are more likely to arise and contribute to evolution than others. In laboratory stocks of the green alga *Volvox carteri*, for example, new mutations affecting the relationship between the size and number of germ cells correspond to the typical state of these characters in other species of *Volvox* (Koufopanou and Bell 1991).

Mutation may not constrain the rate or direction of evolution very much if many different mutations can generate a particular phenotype, as may be the case when several or many loci affect the trait (polygeny). For example, when different copper-tolerant populations of the monkeyflower *Mimulus guttatus* are crossed, the variation in copper tolerance is greater in the F_2 generation than within either parental population, indicating that the populations differ in the loci that confer tolerance (Cohan 1984).

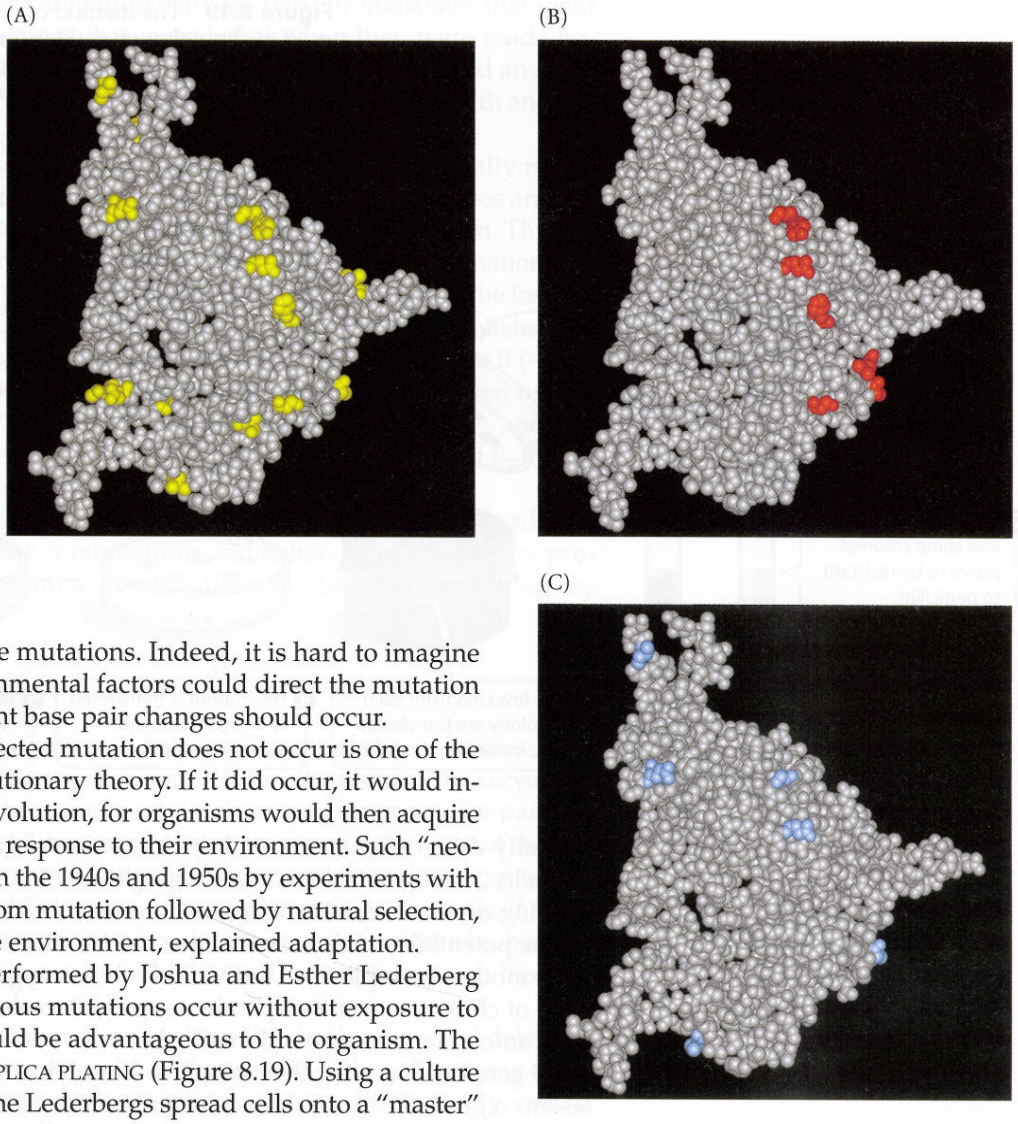
Nevertheless, certain advantageous phenotypes can apparently be produced by mutation at only a very few loci, or perhaps only one. In such instances, the supply of rare mutations might limit the capacity of species for adaptation. The rarity of necessary mutations may help to explain why species have not become adapted to a broader range of environments, or why, in general, species are not more adaptable than they are (Bradshaw 1991). For instance, resistance to the insecticide dieldrin in different populations of *Drosophila melanogaster* is based on repeated occurrences of the same mutation, which, moreover, is thought to represent the same gene that confers dieldrin resistance in flies that belong to two other families (ffrench-Constant et al. 1990). This may mean that very few genes—perhaps only this one—undergo mutations that can confer dieldrin resistance, and that such a mutation is a very rare event.

Wichman et al. (2000) studied experimental populations of the closely related bacteriophage strains ϕX174 and S13 as they adapted to two species of host bacteria at high temperatures. Of the many amino acid substitutions that occurred in the populations, most occurred repeatedly, at a small number of sites, and many of those substitutions matched the variation found in natural populations and even the differences between the two kinds of phage (Figure 8.18). This result suggests that the natural evolution of these phage can take only a limited number of pathways, constrained by the possible kinds of advantageous mutations (Wichman et al. 2000).

Mutation as a Random Process

Mutations occur at random. It is extremely important to understand what this statement does and does not mean. It does not mean that all conceivable mutations are equally likely to occur, because, as we have noted, the developmental foundations for some imaginable transformations do not exist. It does not mean that all loci, or all regions within a locus, are equally mutable, for geneticists have described differences in mutation rates, at both the phenotypic and molecular levels, among and within loci. It does not mean that environmental factors cannot influence mutation rates: as remarked earlier, ultraviolet and other radiation, as well as chemical mutagens, increase rates of mutation.

Mutation *is* random in two senses. First, although we may be able to predict the *probability* that a certain mutation will occur, we cannot predict which of a large number of gene copies will undergo the mutation. The spontaneous process of mutation is stochastic rather than deterministic. Second, and more importantly, mutation is random in the sense that *the chance that a particular mutation will occur is not influenced by whether or not the organism is in an environment in which that mutation would be advantageous.* That is, the en-



igure 8.18 The surface of the ıajor capsid protein (gpF) of hage strains ϕX174 and S13, howing parallel amino acid subtitutions. (A) Amino acids that nderwent substitution in the xperimental lines are shown in ellow. (B) Amino acids shown in ed are known to affect the fitness f wild phage in either of two bacerial host species. (C) Amino cids that represent differences etween the two original strains, X174 and S13, are shown in blue. Note that all the differences that ave naturally evolved between hese phage strains also occurred n the experimental lines. (After Wichman et al. 2000.)

vironment does not induce adaptive mutations. Indeed, it is hard to imagine a mechanism whereby most environmental factors could direct the mutation process by dictating that just the right base pair changes should occur.

The argument that adaptively directed mutation does not occur is one of the fundamental tenets of modern evolutionary theory. If it did occur, it would introduce a Lamarckian element into evolution, for organisms would then acquire adaptive hereditary characteristics in response to their environment. Such "neo-Lamarckian" ideas were expunged in the 1940s and 1950s by experiments with bacteria in which spontaneous, random mutation followed by natural selection, rather than mutation directed by the environment, explained adaptation.

One of these experiments was performed by Joshua and Esther Lederberg (1952), who showed that advantageous mutations occur without exposure to the environment in which they would be advantageous to the organism. The Lederbergs used the technique of REPLICA PLATING (Figure 8.19). Using a culture of *E. coli* derived from a single cell, the Lederbergs spread cells onto a "master" agar plate, without penicillin. Each cell gave rise to a distinct colony. They pressed a velvet cloth against the plate, and then touched the cloth to a new plate with medium containing the antibiotic penicillin, thereby transferring some cells from each colony to the replica plate, in the same spatial relationships as the colonies from which they had been taken. A few colonies appeared on the replica plate, having grown from penicillin-resistant mutant cells. When all the colonies on the master plate were tested for penicillin resistance, those colonies (and only those colonies) that had been the source of penicillin-resistant cells on the replica plate displayed resistance, showing that the mutations had occurred before the bacteria were exposed to penicillin.

Because of such experiments, biologists have generally accepted that mutation is adaptively random rather than directed. However. several investigators have reported results, again with *E. coli*, that at face value seem to suggest that some advantageous mutations might be directed by the environment. Their interpretations have been challenged by other investigators, and there does not appear to be convincing evidence for directed mutation (Sniegowski and Lenski 1995; Brisson 2003).

Recombination and Variation

All genetic variation owes its origin ultimately to mutation, but in the short term, a great deal of the genetic variation within populations arises through recombination. In sexually reproducing eukaryotes, genetic variation arises from two processes: the union of ge-

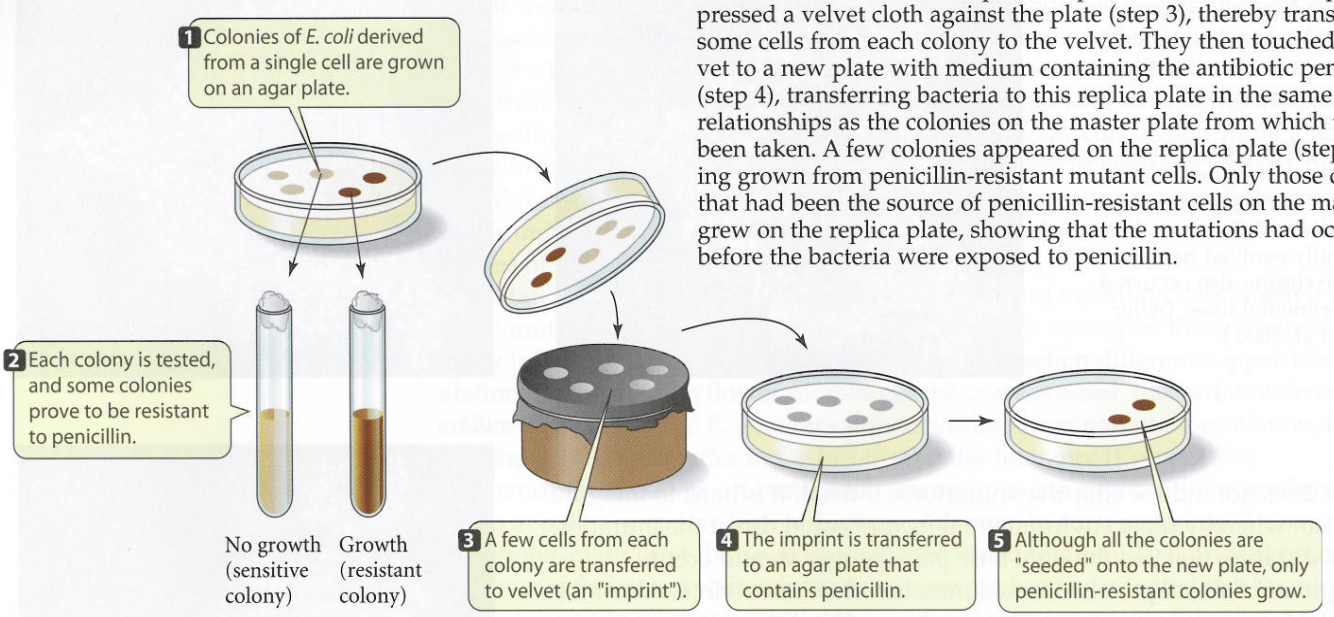


Figure 8.19 The method of replica plating, which the Lederbergs used to show that mutations for penicillin resistance arise spontaneously, before exposure to penicillin, rather than being induced by that exposure. They began (step 1) with an agar plate containing numerous colonies of *E. coli*, all derived from a single cell. They tested the colonies on this master plate for penicillin resistance (step 2). They pressed a velvet cloth against the plate (step 3), thereby transferring some cells from each colony to the velvet. They then touched the velvet to a new plate with medium containing the antibiotic penicillin (step 4), transferring bacteria to this replica plate in the same spatial relationships as the colonies on the master plate from which they had been taken. A few colonies appeared on the replica plate (step 5), having grown from penicillin-resistant mutant cells. Only those colonies that had been the source of penicillin-resistant cells on the master plate grew on the replica plate, showing that the mutations had occurred before the bacteria were exposed to penicillin.

netically different gametes, and the formation of gametes with different combinations of alleles, owing to independent segregation of nonhomologous chromosomes and to crossing over between homologous chromosomes.

The potential genetic variation that can be released by recombination is enormous. To cite a modest example: if an individual is heterozygous for only one locus on each of five pairs of chromosomes, independent segregation alone generates $2^5 = 32$ allele combinations among its gametes, and mating between two such individuals could give rise to $3^5 = 243$ genotypes among their progeny. If each locus affects a different feature, this represents a great variety of character combinations. If all five loci have equal and additive effects on a single polygenic character, such as size, the range of variation among the offspring can greatly exceed the difference between the parents. For instance, if each substitution of + and – alleles in a genotype adds or subtracts one unit of phenotype, two quintuply heterozygous parents (both +–+–+/–+–+–), both of size 20, could have offspring ranging in size from 15 (–/– at all five loci) to 25 (+/+ at all five loci). (Compare the F_1 and F_2 distribution of corolla lengths in Figure 8.12.)

In order to judge how much variation is released by recombination, a team led by the great population geneticist Theodosius Dobzhansky studied the effects of chromosomes they had "extracted" (by a series of crosses; see Figure 9.8) from a wild population of *Drosophila pseudoobscura* (Spassky et al. 1958). Homozygous chromosomes from natural populations of this species show tremendous variation in their effects on survival from egg to adult (see Figure 9.9). However, the Dobzhansky team chose 10 homologous chromosomes that conferred almost the same, nearly normal viability when homozygous and made all possible crosses between flies bearing those chromosomes. From the F_1 female offspring, in which crossing over had occurred, they then extracted recombinant chromosomes and measured their effect on viability when homozygous. Even though the original 10 chromosomes had differed little in their effect on viability, the variance in viability among the recombinant chromosomes was more than 40 percent of the variance found among homozygotes for much larger samples of chromosomes from natural populations. Thus a single episode of recombination among just 10 chromosomes generates a large fraction of a wild population's variability. Some of the recombinant chromosomes were

"synthetic lethals," meaning that recombination between two chromosomes that yield normal viability produced chromosomes that were lethal when they were made homozygous. This finding implies that each of the original chromosomes carried an allele that did not lower viability on its own, but did cause death when combined with another allele, at another locus, on the other chromosome.

Recombination can both increase and decrease genetic variation. In sexually reproducing populations, genes are transmitted to the next generation, but genotypes are not: they end with organisms' deaths, and are reassembled anew in each generation. Thus an unusual, favorable gene combination may occasionally arise through recombination, but if the individuals bearing it mate with other members of the population, it will be lost immediately by the same process. Likewise, if some individuals have mostly + alleles that increase body size (+++/+++) and others have mostly – alleles that decrease it (– – –/– – –), the population will display considerable variation in body size. But, given recombination, most offspring will inherit mixtures of + and – alleles (e.g., +–+/–– +), and will have fairly similar intermediate sizes. (Compare the P generation with F_1 and F_2 in Figure 8.12.)

Recombination, therefore, has complicated effects on variation: it both retards adaptation by breaking down favorable gene combinations and enhances adaptation by providing natural selection with multitudinous combinations of alleles that have arisen by mutation.

Alterations of the Karyotype

An organism's **karyotype** is the description of its complement of chromosomes: their number, size, shape, and internal arrangement. In considering alterations of the karyotype, it is important to bear in mind that the loss of a whole chromosome, or a major part of a chromosome, usually reduces the viability of a gamete or an organism because of the loss of genes. Furthermore, a gamete or organism often is inviable or fails to develop properly if it has an **aneuploid**, or "unbalanced," chromosome complement—for example, if a normally diploid organism has three copies of one of its chromosomes. (For instance, humans with three copies of chromosome 21, a condition known as Down syndrome or trisomy-21, have brain and other defects.)

As we have seen, chromosome structure may be altered by duplications and deletions, which change the amount of genetic material (see Figure 8.5). Other alterations of the karyotype are changes in the number of whole sets of chromosomes (**polyploidy**) and rearrangements of one or more chromosomes.

Polyploidy

A diploid organism has two entire sets of homologous chromosomes ($2N$); a polyploid organism has more than two. (In discussing chromosomes, N refers to the number of different chromosomes in the gametic, or haploid, set, and the numeral refers to the number of representatives of each autosome.) Polyploids can be formed in several ways, especially when failure of the reduction division in meiosis produces diploid, or unreduced, gametes (Ramsey and Schemske 1998). The union of an unreduced gamete (with $2N$ chromosomes) and a reduced gamete (with N chromosomes) yields a TRIPLOID ($3N$) zygote. Triploids produce few offspring because most of their gametes have aneuploid chromosome complements. At segregation, each daughter cell may receive one copy of certain chromosomes and two copies of certain others (Figure 8.20A). However, tetraploid ($4N$) offspring can be formed if an unreduced ($3N$) gamete of a triploid unites with a normal gamete (N) of a diploid—or if two diploid gametes, whether from triploid or diploid parents, unite. Other such unions can form hexaploids ($6N$), octoploids ($8N$), or genotypes of even higher ploidy.

Each set of four homologous chromosomes of a tetraploid may be aligned during meiosis into a quartet (quadrivalent), and then may segregate in a balanced (two by two) or unbalanced (one by three) fashion (Figure 8.20B). In some such polyploids, aneuploid gametes may result and fertility may be greatly reduced. In other cases, the four chromosomes align not as a quartet, but as two pairs that segregate normally, resulting in bal-

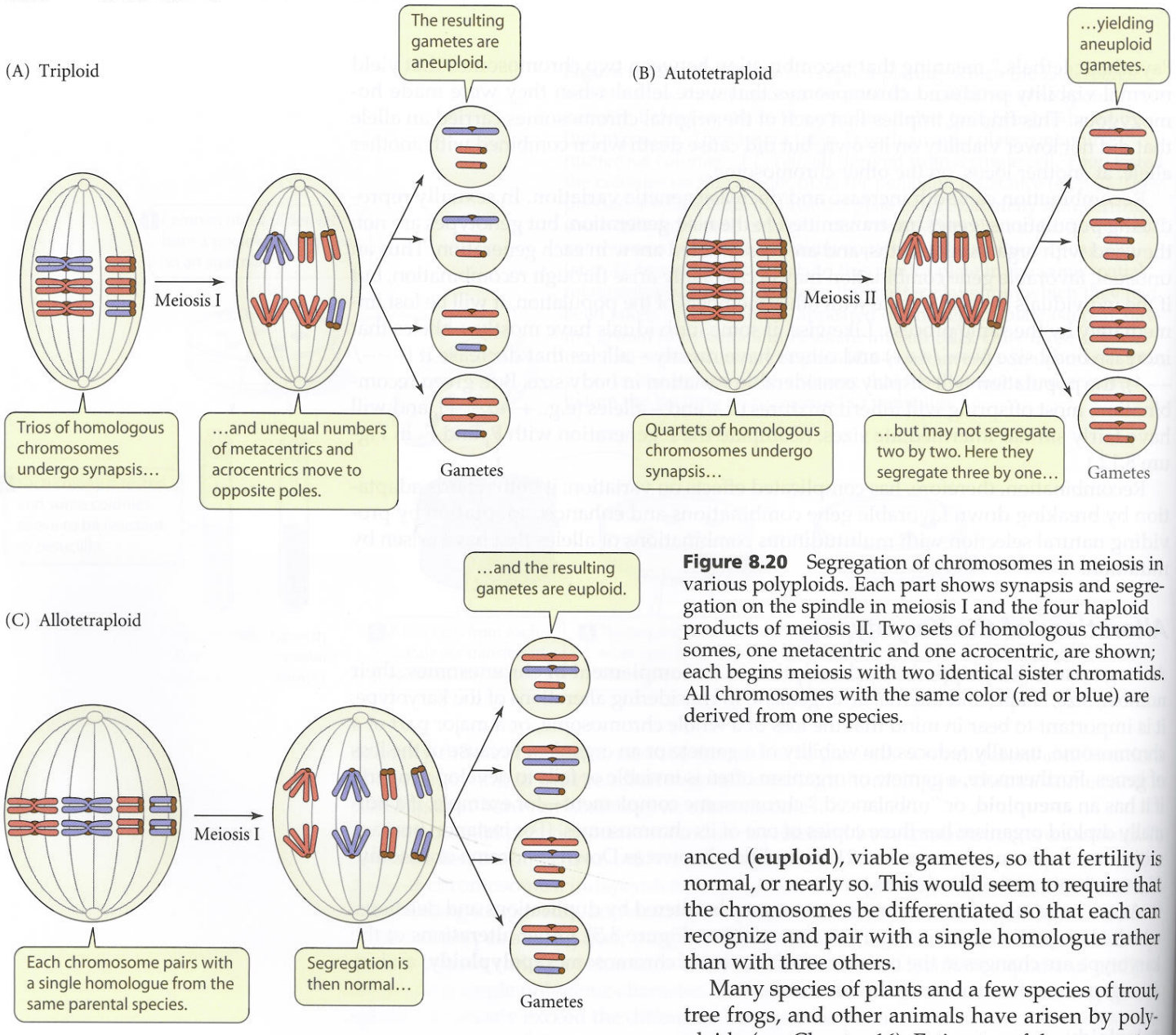


Figure 8.20 Segregation of chromosomes in meiosis in various polyploids. Each part shows synapsis and segregation on the spindle in meiosis I and the four haploid products of meiosis II. Two sets of homologous chromosomes, one metacentric and one acrocentric, are shown; each begins meiosis with two identical sister chromatids. All chromosomes with the same color (red or blue) are derived from one species.

anced (**euploid**), viable gametes, so that fertility is normal, or nearly so. This would seem to require that the chromosomes be differentiated so that each can recognize and pair with a single homologue rather than with three others.

Many species of plants and a few species of trout, tree frogs, and other animals have arisen by polyploidy (see Chapter 16). Estimates of the proportion of angiosperms that are polyploid range up to 50–70 percent (Stace 1989). Some recently arisen polyploids have arisen by the union of unreduced gametes of the same species; these organisms are known as **autopolyploids**. But the majority are **allopolyploids**, which have arisen by hybridization between closely related species. In allopolyploids, most of the parental species' chromosomes are different enough for the chromosomes from each parent to recognize and pair with each other, so that meiosis in an allotetraploid, for example, involves normal segregation of pairs rather than quartets of chromosomes (Figure 8.20C).

Chromosome rearrangements

Changes in the structure of chromosomes constitute another class of karyotypic alterations. These changes are caused by breaks in chromosomes, followed by rejoining of the pieces in new configurations. Some such changes can affect the pattern of segregation in meiosis, and therefore affect the proportion of viable gametes. Although most chromosome rearrangements seem not to have direct effects on morphological or other phenotypic features, an alteration of gene sequence sometimes brings certain genes under the influence of the control regions of other genes, and so alters their expression. It is not certain that such "position effects" have contributed to evolutionary change. Individual or-

ganisms may be homozygous or heterozygous for a rearranged chromosome, and are sometimes referred to as **homokaryotypes** or **heterokaryotypes** respectively.

INVERSIONS. Consider a segment of a chromosome in which ABCDE denotes a sequence of markers such as genes. If a loop is formed, and breakage and reunion occur at the point of overlap, a new sequence, such as ADCBE, may be formed. (The inverted sequence is underlined.) Such an **inversion**, with a rearranged gene order, is PERICENTRIC if it includes the centromere and PARACENTRIC if it does not.

During meiotic synapsis in an inversion heterozygote, alignment of the genes on the normal and inverted chromosomes requires the formation of a loop, which can sometimes be observed under the microscope (Figure 8.21A). Now suppose that in a paracentric inversion, crossing over occurs between loci such as B and C (Figure 8.21B). Two of the four strands are affected. One strand lacks certain gene regions (A), and also lacks a centromere; it will not migrate to either pole, and is lost. The other affected strand not only lacks some genetic material, but also has two centromeres, so the chromosome breaks when these centromeres are pulled to opposite poles. The resulting daughter cells lack certain gene regions, and will not form viable gametes. Consequently, in inversion heterokaryotypes (but not in homokaryotypes), *fertility is reduced* because many gametes are inviable, and *recombination is effectively suppressed* because gametes carrying the recombinant chromosomes, which lack some genetic material, are inviable.

In *Drosophila* and some other flies (Diptera), however, the incomplete recombinant chromosomes enter the polar bodies during meiosis, so that female fecundity is not reduced. It is particularly easy to study inversions in *Drosophila* and some other flies because the larval salivary glands contain giant (polytene) chromosomes that remain in a state of permanent synapsis (so that inversion loops are easily seen), and because these chromosomes display bands, each of which apparently corresponds to a single gene. The banding patterns are as distinct as the bar codes on supermarket products, so an experienced investigator can identify different sequences. INVERSION POLYMORPHISMS are common in *Drosophila*—more than 20 different arrangements of chromosome 3 have been described for *Drosophila pseudoobscura*, for example—and they have been extensively studied from both population genetic and phylogenetic points of view.

TRANSLOCATIONS. By breakage and reunion, two nonhomologous chromosomes may exchange segments, resulting in a **reciprocal translocation** (Figure 8.22). Meiosis in a translocation heterokaryotype often results in a high proportion of aneuploid gametes, so the fertility of translocation heterokaryotypes is often reduced by 50 percent or more. Consequently, polymorphism for translocations is rare in natural populations. Nevertheless, related species sometimes differ by translocations, which have the effect of moving groups of genes from one chromosome to another. The Y chromosome of the male *Drosophila miranda*, for ex-

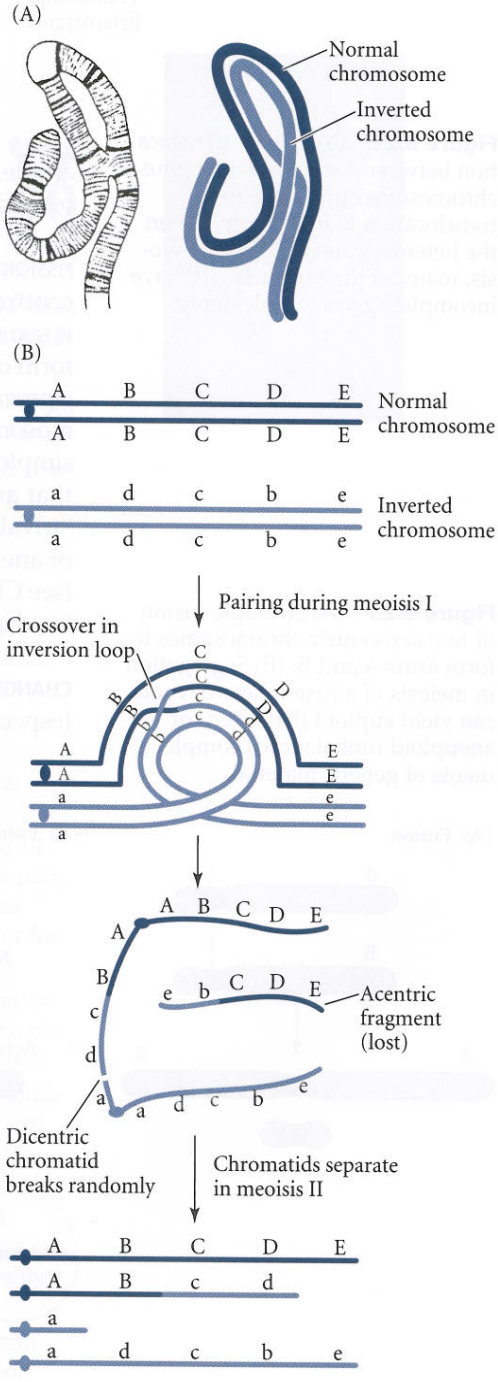


Figure 8.21 Chromosome inversions. (A) Synapsed chromosomes in a salivary gland cell of a larval *Drosophila pseudoobscura* heterozygous for *Standard* and *Arrowhead* arrangements. The two homologous chromosomes are so tightly synapsed that they look like a single chromosome. The "bridge" forms the loop shown in the diagram. Similar synapsis occurs in germ line cells undergoing meiosis. (B) Two homologous chromosomes differing by an inversion of the region B–D, and their configuration in synapsis. Crossing over between two chromatids (between B and C) yields products that lack a centromere or substantial blocks of genes. Because these products do not form viable gametes, crossing over appears to be suppressed. Only cells that receive the two chromatids that do not cross over become viable gametes. (After Strickberger 1968.)

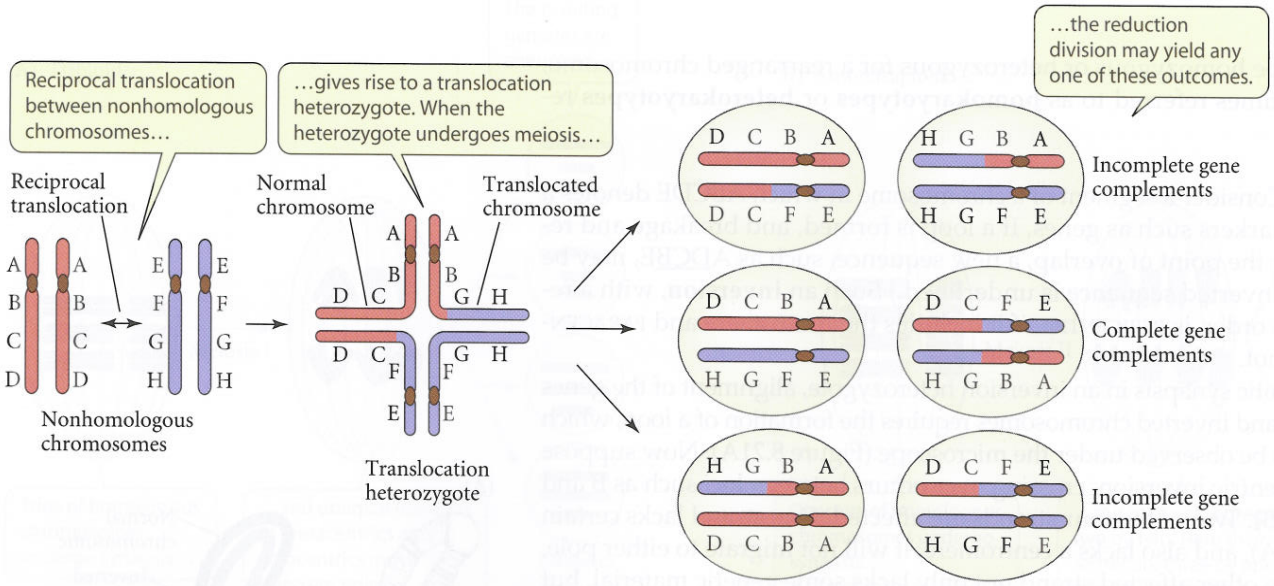


Figure 8.22 Reciprocal translocation between two nonhomologous chromosomes gives rise to a translocation heterozygote. When the heterozygote undergoes meiosis, many of the products will have incomplete gene complements.

ample, includes a segment that is homologous to part of one of the autosomes of closely related species.

FISSIONS AND FUSIONS. It is useful to distinguish ACROCENTRIC chromosomes, in which the centromere is near one end, from METACENTRIC chromosomes, in which the centromere is somewhere in the middle and separates the chromosome into two arms. In the simplest form of chromosome FUSION, two nonhomologous acrocentric chromosomes undergo reciprocal translocation near the centromeres so that they are joined into a metacentric chromosome (Figure 8.23A). More rarely, a metacentric chromosome may undergo **fission**. A simple fusion heterokaryotype has a metacentric, which we may refer to as AB, with arms that are homologous to two acrocentrics A and B. AB, A, and B together synapse as a "trivalent" (Figure 8.23B). Viable gametes and zygotes are often formed, but the frequency of aneuploid gametes can be quite high, especially for more complex patterns of fusion (see Chapter 16). Differences in chromosome number due to fusions often distinguish related species or geographic populations of the same species.

CHANGES IN CHROMOSOME NUMBER. Summarizing what we have covered so far, polyploidy (especially in plants), translocations, and fusions or fissions of chromosomes are the mu-

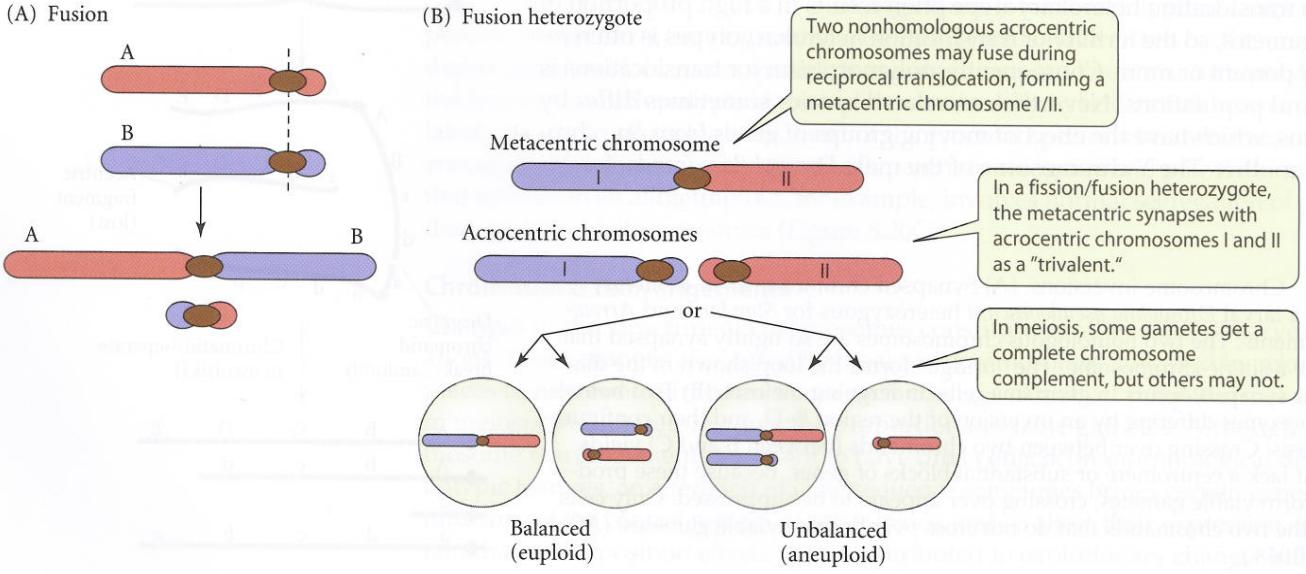


Figure 8.23 (A) A simple fusion of two acrocentric chromosomes to form arms A and B. (B) Segregation in meiosis of a fusion heterozygote can yield euploid (balanced) or aneuploid (unbalanced) complements of genetic material.

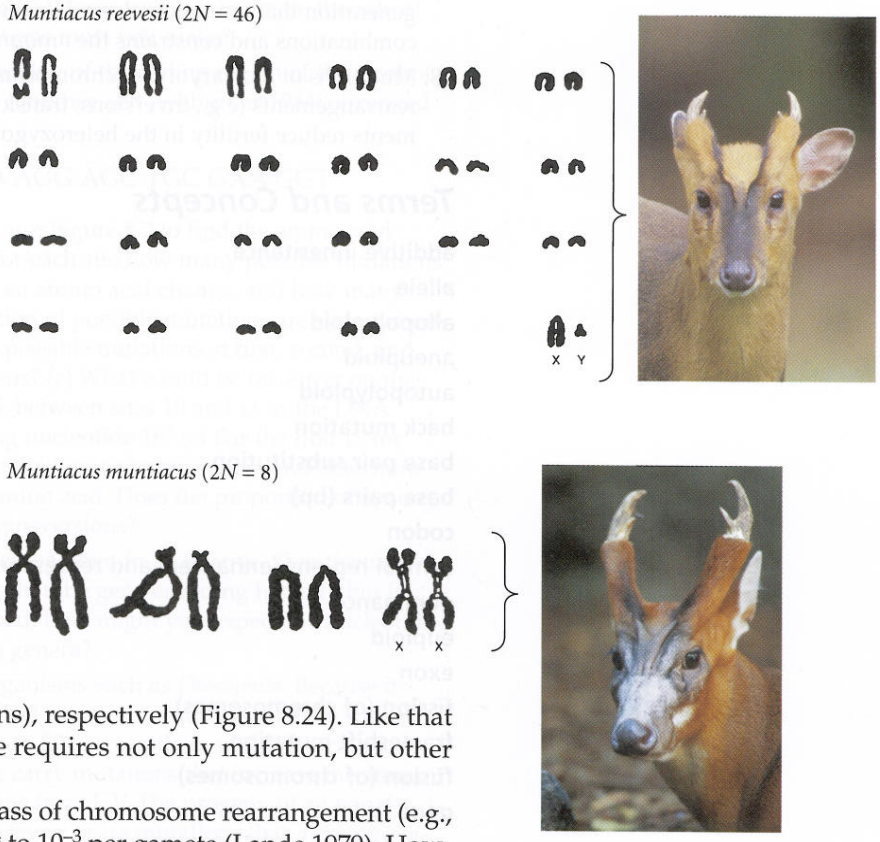


Figure 8.24 The diploid chromosome complements (taken from micrographs) of two closely related species of muntjacs (barking deer) represent one of the most extreme differences in karyotype known among closely related species. Despite the difference in karyotype, the species are phenotypically very similar. (*M. reevesii* photo © Mike Lane/Alamy Images; *M. muntiacus* © OSF/photolibrary.com)

tational foundations for the evolution of chromosome number. For example, the haploid chromosome number varies among mammals from 3 to 42 (Lande 1979), and among insects from 1 in an ant species to about 220 in some butterflies (the highest number known in animals). Related species sometimes differ strikingly in karyotype: in one of the most extreme examples, two very similar species of barking deer, *Muntiacus reevesii* and *M. muntiacus*, have haploid chromosome numbers of 23 and 3 or 4 (in different populations), respectively (Figure 8.24). Like that of all characteristics, evolution of the karyotype requires not only mutation, but other processes as well (see Chapter 16).

The spontaneous rate of origin of any given class of chromosome rearrangement (e.g., reciprocal translocation) is quite high: about 10^{-4} to 10^{-3} per gamete (Lande 1979). However, a rearrangement involving breakage at any particular site(s) rarely arises, and is usually considered to be unique.

Summary

1. Mutations of chromosomes or genes are alterations that are subsequently replicated. They ordinarily do not constitute new species, but rather variant chromosomes or genes (alleles, haplotypes) within a species.
2. At the molecular level, mutations of genes include base pair substitutions, frameshifts, duplications and deletions of one or more base pairs (or of longer sequences that may include entire genes), and changes caused by insertion of various kinds of transposable elements. New DNA sequences also arise by intragenic recombination.
3. The rate at which any particular mutation arises is quite low: on average, about 10^{-6} to 10^{-5} per gamete for mutations detected by their phenotypic effects, and about 10^{-9} per base pair. The mutation rate, by itself, is too low to cause substantial changes of allele frequencies. However, the total input of genetic variation by mutation, for the genome as a whole or for individual polygenic characters, is appreciable.
4. The magnitude of change in morphological or physiological features caused by a mutation can range from none to drastic. In part because most mutations have pleiotropic effects, the *average* effect of mutations on fitness is deleterious, but some mutations are advantageous.
5. Mutations alter pre-existing biochemical or developmental pathways, so not all conceivable mutational changes are possible. Some adaptive changes may not be possible without just the right mutation of just the right gene. For these reasons, the rate and direction of evolution may in some instances be affected by the availability of mutations.
6. Mutations appear to be random, in the sense that their probability of occurrence is not directed by the environment in favorable directions, and in the sense that specific mutations cannot be predicted. The likelihood that a mutation will occur does not depend on whether or not it would be advantageous.
7. Recombination of alleles can potentially give rise to astronomical numbers of gene combinations, and in sexually reproducing organisms generates far more genetic variation per