Stearns & Hockstra
PP 99-108
and 119-120

CHAPTER 5

The origin and maintenance of genetic variation

Without genetic variation, there can be no evolution

● **KEY CONCEPT**

The amount of genetic variation affecting fitness limits the response to selection.

Chapter 4 discussed the genetic response to selection, and it assumed that genetic variation was present. If all individuals in a population were genetically identical and produced offspring identical to themselves, evolutionary change would be impossible. Both adaptive and neutral evolution require heritable differences among individuals to change the genetic composition of a population. Genetic variation is essential for evolutionary change.

It is not just the presence but the amount of genetic variation that influences the rate of evolutionary change. If there were very little genetic variation, the rate of evolutionary change would be limited by rare favorable variants. Most individuals would have a standard set of genes (termed *wild type* by the classical geneticists). Natural selection would remove deleterious variants, and occasionally a favorable variant would spread through the population. If, in contrast, there is a great deal of genetic variation, as we now know is usually the case, then individuals will differ genetically in many traits. Classification of most of the population as wild type is not possible, and the rate of evolutionary change is not limited by the occurrence of new favorable mutations. In an extreme case, with great genetic variability associated with great variability in fitness, selection could be too strong, removing such a large fraction of the population each generation due to low survival or fertility that it might go extinct.

Clearly, how much genetic variation is present, how it is maintained, and how much of it is correlated with fitness are crucial issues. In this chapter we first consider the origin of genetic variation, then its maintenance, and finally its relevance for adaptive evolution.

Mutations are the origin of genetic variation

KEY CONCEPT
The ultimate origin of all genetic variation is mutation.

Mutation: *A hereditary change in the DNA sequence or in chromosome number, form, or structure.*

All genetic variation originates through mutation. The different types of mutation are explained in more detail in the Genetic appendix (pp. 515 ff.). Most mutations arise from errors during DNA replication. Mutations can occur in somatic cells (body cells) as well as in the germ line (cells that end up as eggs and sperm). Somatic mutations can affect the function of individual organisms, both positively and negatively. For example, somatic mutation—together with other mechanisms—helps to generate antibody diversity in the immune system and thus contributes to the defense against pathogens; in contrast, some somatic mutations cause cancer. Only very rarely do mutations enhance reproductive success. An overwhelming majority of both somatic and germ-line mutations are deleterious or neutral. Germ-line mutations are more important for evolution, for unlike somatic mutations they are transmitted to future generations. In plants and fungi, which do not have a germ line, reproductive tissue can develop from somatic cells. Thus in these organisms some somatic mutations are inherited.

While mutations are necessary for evolution, too frequent mutation can prevent evolution, for with a very high mutation rate, not enough of the well-adapted genes would be transmitted unchanged to the next generation. Their loss would prevent the evolution and maintenance of adaptations. Thus there is likely to be an optimal mutation rate: not too few and not too many mutations. This optimal rate need not be the same for all species and all genes. That a population sometimes cannot survive a high mutation rate was shown in an experiment (Zeyl et al. 2001) in which replicate populations of two different yeast strains were propagated. In the strain with a 200-fold enhanced mutation rate, extinction was observed in two out of 12 replicate populations, whereas no extinctions were observed in the 12 populations with a normal mutation rate.

We have good reason to think that the mutation rate is to some extent under genetic control. In several species (mainly microorganisms) genetic variation for the mutation rate has been observed. For example, in bacteria so-called mutator strains are known, which have an enhanced mutation rate due to less-efficient repair of DNA damage. Thus mutation rates can be changed by natural selection.

Optimal mutation rates are easier to achieve in asexual organisms

Sexual and asexual species differ in the ease with which mutation rates can be adjusted by selection. In asexual organisms, where the whole genome is transmitted intact to the offspring, evolution of the mutation rate is easy in principle, for the genes that affect the mutation rate stay together with the genes whose mutation rate they adjust. If conditions favor a higher mutation rate, a mutation that enhances the mutation rate at all loci enjoys a selective advantage and will

increase in frequency because it stays associated with the genotype that is benefiting from the higher mutation rate. In contrast, in sexual organisms a gene affecting mutation rate does not remain associated with the genome on which it has its effect because recombination can separate the gene determining the mutation-rate gene from the genes that mutate. Therefore evolution of the mutation rate to a value that maximizes the rate of adaptive evolution is expected to occur more readily in asexual species than in sexual species.

Rates of mutation

KEY CONCEPT

Mutations rates differ strikingly in DNA and RNA genomes, for mutations of small versus large effects, and in males and females.

The average mutation rate per nucleotide pair per replication is about 10^{-10}—1 in 10 billion—in organisms with DNA genomes (Drake et al. 1998). Some viruses (for example those causing influenza and HIV) have a genome coded in RNA instead of DNA; they have much higher mutation rates, because repair of damage is less efficient in RNA genomes than in DNA genomes. The figure given is only a rough generalization, for mutation rates per nucleotide pair can vary by orders of magnitude among loci and among species. In microorganisms with DNA genomes the mutation rate appears to be strikingly constant when calculated per genome instead of per nucleotide pair: despite huge variations in genome size, all microbes have a mutation rate of approximately 1 in 300 per genome per replication. This implies that the mutation rates per nucleotide pair must also vary considerably. In higher eukaryotes estimates of the mutation rate vary between 0.1 and 100 per genome per generation. This figure is substantially higher than in microbes and probably arises because higher eukaryotes have both much larger genome sizes and many cell divisions per generation. However, the mutation rate in higher eukaryotes per cell division per *effective* genome (the part of the genome that codes for functional genes) may be of the same order of magnitude as in microbes (1 in 300). These numbers are derived from measurements on DNA sequences and do not tell us about the phenotypic consequences of the mutations for the fitness of individuals.

Mutations with large effects are much less frequent than those with small effects

A classical way to determine the likelihood of a mutation is to observe the spontaneous occurrence of abnormal phenotypes known to result from single allele changes. Since in this approach the number of mutations is given as a function of the number of individuals or gametes measured, it is best to call the resulting estimate *mutation frequency* to avoid confusion with the earlier-mentioned *mutation rates*, which are based on numbers of mutations per unit of time (replication or generation). Through the study of spontaneous mutations that cause human diseases mutations have been estimated to occur with a frequency

of about 10^{-5}—1 per 100 000—per gamete. Similar figures have been obtained from studies in mice and *Drosophila*.

Using another approach, *Drosophila* geneticists (Mukai 1964, Mukai et al. 1972, Houle et al. 1992) have accumulated recessive (or partially recessive) mutations over many generations on a chromosome that is kept heterozygous and prevented from recombining. At regular intervals the fitness effect of the chromosome is measured in homozygotes, where the recessive mutations are expressed. The results suggest that the mutation frequency in *Drosophila* is about one mutation with a small deleterious effect per zygote and that mildly deleterious mutations greatly outnumber lethal ones.

Because humans have much more DNA than *Drosophila* and mutation rates per locus per generation are similar in humans and *Drosophila*, an average human might carry tens of new mutations, but many of them would be in DNA that did not code for proteins.

More mutations occur in males than in females

Recent molecular data on human genetic diseases suggests higher point-mutation rates in males than in females in some genes. Extreme examples are achondroplasia and Apert Syndrome, two dominantly inherited disorders. In both, all new mutations occurred in the father in more than 50 cases. The higher male point-mutation rate may be related to the much higher number of cell divisions in the male than in the female germ line (Crow 1997). That point mutations are associated with cell division makes this explanation plausible.

How random are mutations?

● KEY CONCEPT

The effects of mutations have no systematic relationship to the needs of the organism.

It is often stated that natural selection produces adaptations by acting on variation resulting from random mutations. What does the word random mean in this context? Because some parts of a genome experience much higher rates of mutations than other parts, mutation is not random with respect to where it occurs. Mutations can also be triggered by a specific signal, for example, in the fungus *Neurospora crassa*, where newly duplicated sequences trigger a specific mutational response (called RIP) that deactivates the repeated sequence (Selker 1990). RIP is an adaptive mutation, for it prevents the harmful accumulation of non-functional repeated sequences. Enhanced mutation rates at places in the genome where a high level of genetic variability is advantageous are also adaptive. Examples include the high level of somatic mutation in immune receptor genes in the vertebrate immune system and the highly mutable bacterial genes involved in the interactions of pathogenic bacteria with their hosts (Moxon et al. 1994). Mutations do not occur at random with

respect to their location in the genome. Some genes mutate more frequently than others.

The critical question, however, is this: do mutations with a specific phenotypic effect occur more often when they are advantageous than when they are not? If so, adaptations could be produced by mutation alone, and natural selection would be less important. Such a directed mutational process is called Lamarckian because it resembles Lamarck's idea that an adaptation acquired by an organism during its lifetime can be transmitted to its offspring (see Chapter 1, pp. 13, 15 ff. for Lamarck's role in the history of evolutionary thinking). This would be the case, for example, if an animal could transmit to its offspring the immunity to a disease that it had developed through an immune response—but it cannot. We cannot at present rule out Lamarckian mutations entirely, but there is no evidence for them at the level of genetic mutations (changes in DNA sequence), and there is no evidence that they are very important.

Mutations, on the other hand, are certainly random in the sense that there is no systematic relationship between their phenotypic effect and the actual needs of the organism in which they occur. Note that it is the *specific* phenotypic effect of a mutation that matters here. Vertebrates require antibody diversity to produce an effective immune response, and a mutational process helps generate this diversity. However, this is not a Lamarckian process because the presence, for example, of influenza virus does not affect the probability that a somatic mutation yields resistance to influenza virus.

DNA duplication events increase the number of genes, providing a substrate for the evolution of new functions

Mutations that change the amount of DNA or the number of genes are key events in evolution. They include polyploidization and duplication of genes or gene clusters. Polyploidization—together with other mutations affecting the number and structure of chromosomes—is the main process responsible for differences in the number of chromosomes and in the total amount of DNA among species. Polyploidy is found in many plants and is thought to have played a very important role in the origin of new species (see Chapter 12, pp. 293 ff.). It is also common in certain vertebrate groups, such as the salmonid fishes and frogs. DNA duplication increases the total amount of DNA in the genome, providing material for the evolution of new functions. Its role has been substantial in evolution. For example, recent analyses of the human genome have revealed that over 15% of human genes are duplicates (Li et al. 2001). Duplicate copies of genes will accumulate mutations independently and may diverge to acquire a new function. Several mechanisms have been proposed to explain how such functional divergence between duplicated genes may work (Prince and Pickett 2002). In addition to DNA duplication, other mechanisms have been discovered that increase the size of the genome.

Polyploidization: *A doubling of the complete chromosome set.*

Duplication: *Copying of a DNA sequence without loss of the original, increasing the size of the genome by the size of the sequence copied.*

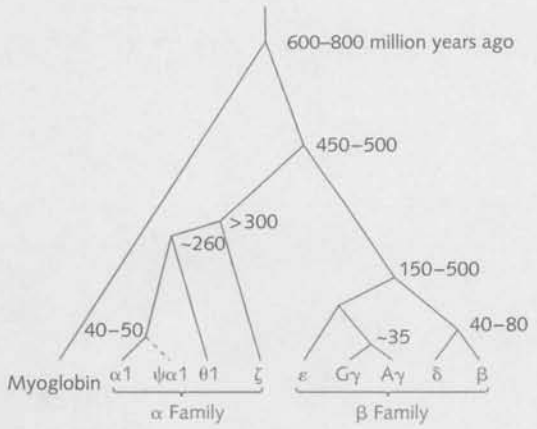

Figure 5.1 Phylogenetic tree of human globin genes, illustrating a series of gene duplications. The human globin gene family consists of three groups, the myoglobin gene on chromosome 22, the α-globin genes on chromosome 16, and the β-globin genes on chromosome 11. Several pseudogenes, remnants of duplicated genes that have become nonfunctional due to mutations, are in this gene family. Hemoglobin has two protein chains, one coded by a gene from the α and one by a gene from the β group. The various combinations differ in oxygen-binding affinities and appear at different developmental stages (embryo, fetus, adult). (From Li and Graur 1991.)

Repeated gene duplication produces multigene families

There are many *multigene families*, consisting of genes that have arisen by duplication from a common ancestral gene and have retained similar function. Examples in mammals include genes coding for heat-shock proteins (involved in protection of cells against environmental stress), globin proteins (involved in oxygen transport), apolipoproteins (involved in lipid metabolism), oncogenes (implicated in cancer), *HOX* genes (very important in development; discussed in Chapter 6, pp. 137 ff.), and genes involved in the immune system. Figure 5.1 depicts the evolutionary history of the human globin genes. An ancient duplication allowed divergence into two types of functional globin protein: myoglobin, for oxygen storage in muscles, and hemoglobin, for oxygen transport in blood.

Further duplications and divergence have produced the α and β families of hemoglobin, which consist of functional genes, like α1, θ1, and ζ in the α family and ε, γ, δ, and β in the β family, and pseudogenes, nonfunctional remnants of once functional genes, such as ψα1 in the α family.

The effect of recombination on genetic variability

● **KEY CONCEPT**

Recombination during meiosis creates great genetic diversity among offspring.

Because of recombination during meiosis, sexual individuals produce haploid gametes that differ genetically from the gametes that formed them. Thus an *AaBb* individual, grown from a zygote that resulted from the fusion of an *AB*

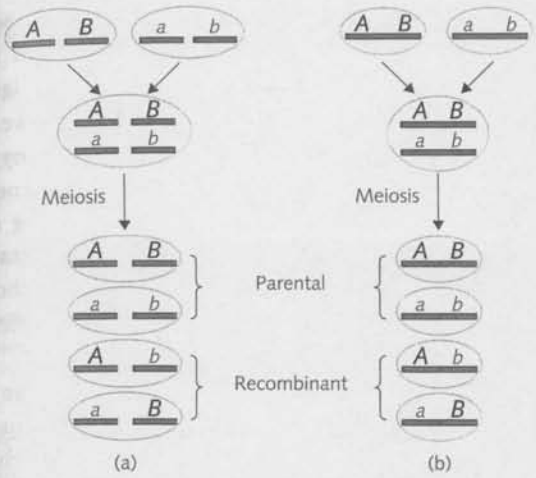

Figure 5.2 Recombination between loci on different chromosomes may result from Mendelian independent assortment (a) and recombination between loci on the same chromosome from crossing over (b).

and an *ab* gamete, will produce gametes both with the parental haplotypes *AB* and *ab* and with the recombinant haplotypes *Ab* and *aB* (Figure 5.2).

Haplotypes: *Groups of closely linked genes that tend to be inherited together.*

When two DNA sequences are located on different chromosomes, they segregate independently at meiosis. When they are on the same chromosome, they segregate together unless a crossover occurs between them. In either case recombination is between genes and may result in new combinations of genes on a chromosome or in an individual. Thus recombination affects genetic variation among individuals when combinations of many loci are considered. Only rarely does it affect variation at a single locus.

Recombination produces phenotypes well outside the starting range

The effectiveness of recombination at converting potential into actual variability is spectacular in animal and plant breeding, where, starting from a uniform population established by crossing two inbred lines, individuals can be selected in a few generations with traits well outside the range of the original population. The examples mentioned in Chapter 4 (p. 93) of large and rapid phenotypic change under directional selection in *Drosophila* and in dogs illustrate this point. Selection can be so effective because the traits are affected by many genes whose recombination generates many combinations of alleles across multiple loci.

The amount of genetic variation in natural populations

The amount of genetic variation affecting fitness is important to know but hard to measure

● **KEY CONCEPT**

Molecular methods have revealed tremendous genetic variation in natural populations.

To understand adaptive evolution, we must know how much genetic variation there is in natural populations and how much of it affects individual reproductive success. This has been one of the major questions of population

genetics since about 1920. Attempts to answer this question have been hampered by two related problems. Both stem from our ignorance of the relationships between genotypes and phenotypes. First, except where large phenotypic differences show Mendelian segregation patterns in crosses, we do not know the genetic variation that underlies the observable phenotypic variability. Second, we can only measure fitness effects of individual genetic variations when they are fairly large. The first problem—not knowing the genetic variation underlying phenotypic differences—was the main obstacle to estimating the amount of natural genetic variation until molecular methods were introduced in the 1960s. Then the problem of measuring fitness effects took priority.

In the mid-1960s biologists started to apply the biochemical technique of enzyme electrophoresis to samples of individuals from natural populations of animals and plants. Electrophoresis separates proteins on the basis of their mobility through a gel under the influence of an electric current. Proteins that differ in their net electrical charge move at different speeds. This can be observed by staining the proteins after they have moved through the gel for some time. The technique greatly improved estimates of genetic variation, for it made visible the variation at loci that could until then not be inferred from the phenotypes. The amount of genetic variability in populations is usually measured by the *genetic diversity*, h, defined as the probability that two alleles chosen at random from all alleles at that locus in the population are different. The easiest way to compute this probability is by seeing that it equals 1 minus the probability that two randomly chosen alleles are identical. If the relative frequency of allele A_1 is x_1, the probability that two randomly chosen alleles are both A_1 equals x_1^2. This applies similarly for all other alleles in the population. Therefore the probablity that two randomly chosen alleles are the same is the sum of the probabilities for each allele separately. Denoting the frequency of allele i by x_i, we get

$$h = 1 - \sum_i x_i^2 \qquad [5.1]$$

So, when a population has little genetic variation, the probability of two alleles being identical is high, and h will be close to 0. If, on the other hand, there is much genetic variability, the probability of two alleles being identical is low, and h will be high. When the population mates randomly, the two alleles at a locus in an individual form a random pair. Therefore under random mating the genetic diversity h equals the actual *heterozygosity*, H, the proportion of the population that is heterozygous at a locus. You can check with eqn 5.1 that for the case of two alleles $h = 2x_1x_2$, which is the familiar Hardy–Weinberg frequency of heterozygotes. If we average over loci, H can also be interpreted as the average proportion of loci that are heterozygous per individual.

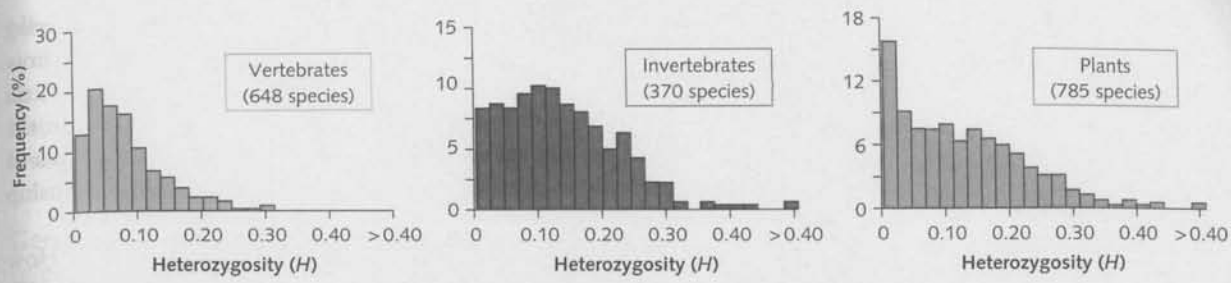

Figure 5.3 Estimates of heterozygosity, H, based on protein-electrophoretic surveys in many different species. (From Avise 1994.)

Electrophoretic heterozygosity is about 10% and varies among populations and species

Protein electrophoresis has been applied to many samples from populations of many species. The results of many studies suggest that H is about 10% and varies between populations and species (Figure 5.3). Such high levels of genetic variability were unexpected and, as we shall discuss later in this chapter, stimulated the development of theories that could explain the stable maintenance of all this variation.

Since 1980 more-refined molecular techniques have yielded measurements of genetic variability at higher resolution. One approach is to isolate DNA and cut it with restriction enzymes that recognize particular short nucleotide sequences. The resulting DNA fragments can be separated by gel electrophoresis according to molecular weight and visualized as stained bands. Differences between homologous chromosomes in the location of restriction sites (the short nucleotide sequences recognized by the restriction enzymes) can thus be measured. Another approach is to sequence the DNA to get the nucleotide sequence itself (e.g. . . . AATGCTTCGA . . .). This became practical with the development of the polymerase chain reaction (PCR), which amplifies small amounts of DNA, even the DNA from a single cell.

Nucleotide diversities are about 0.0001–0.01 within populations

Both restriction analysis and sequencing allow us to estimate genetic variability at the level of nucleotides. The genetic diversity h is not a good measure of the variability of DNA sequences, for when long homologous sequences are compared all nucleotide sequences differ from each other, and h is close to 1. A better measure is the *nucleotide diversity*, the average number of nucleotide differences per site between randomly chosen pairs of sequences. Nucleotide diversities are typically in the range 0.0001–0.01.

Molecular methods do not *solve* the problem of deducing the underlying genetic variation from observed phenotypic variability: they *circumvent* it.

Molecular methods give direct access to genomic information without using phenotypic variation to draw conclusions about the genotype. They tell us how much genetic variation is present in a particular part of the genome, but they do not tell us how this genetic variation affects phenotypic variation. In a sense, the patterns of stained bands on a gel representing protein variants or pieces of DNA are phenotypes made visible by molecular techniques, but the relationship of those bands to fitness is rarely clear.

Thus molecular methods have revealed enormous genetic variation. How much of this variation causes fitness variation and serves as a substrate for adaptive evolution? Or, to put the same question the other way round, how much molecular genetic variation is selectively neutral? This is an empirical question. In Chapter 3 we discussed Kimura's neutral theory (p. 66), which claims that most variation at the molecular level is neutral. His theory caused considerable controversy about the relative importance of genetic drift and adaptive evolution in molecular evolution. We next discuss some attempts to measure fitness consequences of molecular genetic variation. Then we consider some models that aim to understand how mutation, genetic drift, and natural selection affect the level of genetic variation in a population.

Evidence of natural selection from DNA sequence evolution

Functionally important sites in DNA molecules experience natural selection

● **KEY CONCEPT**
Both selection and drift have played important roles in the evolution of DNA sequences.

No one believes that all genetic variation is selectively neutral. The abundant evidence of adaptation through natural selection (Chapter 2, pp. 35 ff.) must be reflected in DNA sequences. The question is how much of the variation in DNA sequences can be considered neutral. In a few cases strong indirect evidence of adaptive evolution has been obtained from comparisons of homologous DNA sequences. For example, Hughes and Nei (1989) compared the DNA sequences of the antigen-recognition sites of major histocompatibility (MHC) genes of humans and mice, genes involved in immune responses (their name refers to the role these genes play in the rejection of organ transplants or tissue grafts). Rates of substitution were estimated by counting the number of nucleotide differences between homologous stretches of DNA, and synonymous and non-synonymous substitution rates were distinguished. Non-synonymous substitutions change the amino acid coded; synonymous substitutions do not. Synonymous changes are usually more frequent than non-synonymous ones because amino acid replacements often reduce protein function and are selected against. Based on 36 protein-coding genes, the mean rate of synonymous substitution had been estimated to be five times higher than for non-synonymous substitution (Li and Graur 1991). Hughes and

continuously on the same culture medium with glucose as the sole carbon and energy input. Thus in this experiment the whole process could be followed starting from the occurrence of a novel mutation until the establishment of a stable allele frequency equilibrium. Rozen and Lenski showed that *S* had originated by mutation around generation 4000.

Genetic diversity of complex quantitative traits

Stabilizing selection is common in quantitative traits

● **KEY CONCEPT**

Quantitative traits are usually under stabilizing selection, and genetic variation for such traits is probably maintained by the mutation–selection balance.

Many quantitative characters are under stabilizing selection: some intermediate trait value is the best, smaller and larger values reduce function. The general reason is probably tradeoffs between different functions of a trait: a higher metabolic rate provides more potential for growth and activity, but a lower rate requires less food and resources; stronger bones give better support for the body, but lighter bones are cheaper to make and require less energy to carry; higher blood pressure promotes rapid transport of substances via the bloodstream, but lower blood pressure is better for the vascular system; laying more eggs means more potential offspring, but laying fewer eggs costs less and allows better care of each offspring.

If artificial selection of wild species can produce big changes both up and down, then natural selection must have been stabilizing. Artificial selection has often demonstrated that genetic variability exists to change body size in both directions: consider the sizes of the different breeds of dogs. Much of the genetic potential for variation in body size in dogs must have been present in wolves, from which dogs descend. Yet wolves have had a constant size for millions of years, which must mean that larger and smaller wolves are selected against.

Thus natural selection often favors some intermediate trait value at which the net benefit of the different functional aspects of the trait is highest. This means that often selection is stabilizing: deviations from the optimum phenotype are selected against. This is even likely to be true when paleontological evidence indicates a long-term directional change. For example, horses have evolved from ancestors that were about the size of a very small pony 50 million years ago, but the mean rate of change was so slow that it can be explained by very weak directional selection or even by genetic drift. Throughout that long history the body size of horses could have often been under stabilizing selection. One of many well-documented examples of stabilizing selection is the birth weight of human babies (Figure 5.10).

Genetic variation for quantitative traits is abundant

There is genetic variation for almost all quantitative traits in natural populations. Plant and animal breeders know that artificial selection on practically any trait

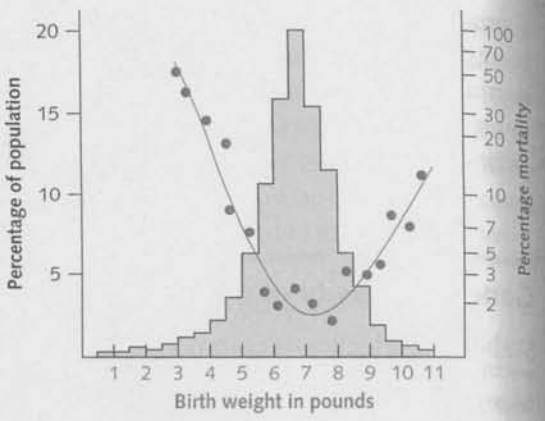

Figure 5.10 The distributions of birth weight (bars) and early mortality (circles) among 13 730 babies. These data (from Karn and Penrose 1951) indicate stabilizing selection on birth weight, since the optimum birth weight is associated with the lowest mortality. (From Cavalli-Sforza and Bodmer 1971.)

will produce a selection response, confirming the presence of genetic variability for the trait. As explained in Chapter 4(pp. 86 ff.), the extent of this genetic variation cannot be expressed in terms of the genetic diversity or heterozygosity at the loci involved, because those loci are almost always unknown. Instead, we can estimate what fraction of the phenotypic variation is due to genetic variation. So although we cannot point to the genes that vary, we know that populations do contain much genetic variation for quantitative traits.

Overdominance and frequency-dependence are not plausible ways to maintain quantitative variation

How is genetic variation maintained under stabilizing selection? In theory, overdominance or negative frequency-dependent selection could be responsible. It is hard to tell as long as the genes involved are not known. On *a priori* grounds it does not seem likely that overdominance predominates at the many loci involved in quantitative traits under stabilizing selection. Because firm evidence for single-locus overdominance is scarce (the sickle-cell polymorphism in malarial areas is one of the few well-documented cases), assuming that it occurs at many loci affecting quantitative traits is unjustified. The *a priori* case for frequency-dependent selection is perhaps stronger. If the optimal phenotype under stabilizing selection is created by roughly equal numbers of + and − alleles, a shortage of, for example, + alleles at the population level will move the average phenotype away from the optimum towards the − side and selection will favor the rare + alleles. But selection pressures on individual alleles are then likely to be very weak (see below), and empirical evidence of the involvement of widespread frequency-dependent selection is lacking.

Mutation–selection balance can maintain quantitative variation

A plausible explanation is the mutation–selection balance. A mutation that slightly increases or decreases an optimal phenotype will experience very weak