Principles of GENETICS

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Chapter 3
Mendelism: The Basic Principles of Inheritance

CHAPTER OUTLINE

- Mendel's Study of Heredity
- Applications of Mendel's Principles
- Testing Genetic Hypotheses
- Mendelian Principles in Human Genetics

Pisum sativum, the subject of Gregor Mendel's experiments.

The Birth of Genetics: A Scientific Revolution

Science is a complex endeavor involving the careful observation of natural phenomena, reflective thinking about these phenomena, and the formulation of testable ideas about their causes and effects. Progress in science often depends on the work of a single insightful individual. Consider, for example, the effect that Nicolaus Copernicus had on astronomy, that Isaac Newton had on physics, or that Charles Darwin had on biology. Each of these individuals altered the course of his scientific discipline by introducing radically new ideas. In effect, they began scientific revolutions.

In the middle of the nineteenth century, the Austrian monk Gregor Mendel, a contemporary of Darwin, laid the foundation for another revolution in biology, one that eventually produced an entirely new science—genetics. Mendel's ideas, published in 1866 under the title "Experiments with Plant Hybrids," endeavored to explain how the characteristics of organisms are inherited. Many people had attempted such an explanation previously but without much success. Indeed, Mendel commented on their failures in the opening paragraphs of his article:

> *To this object, numerous careful observers, such as Kölreuter, Gärtner, Herbert, Lecoq, Wichura and others, have devoted a part of their lives with inexhaustible perseverance....*
>
> *[However], Those who survey the work in this department will arrive at the conviction that among all the numerous experiments made, not one has been carried out to such an extent and in such a way as to make it possible to determine the number of different forms under which the offspring of the hybrids appear, or to arrange these forms with certainty according to their separate generations, or definitely to ascertain their statistical relations.[1]*

He then described his own efforts to elucidate the mechanism of heredity:

> *It requires indeed some courage to undertake a labor of such far-reaching extent; this appears, however, to be the only right way by which we can finally reach the solution of a question the importance of which cannot be overestimated in connection with the history of the evolution of organic forms.*
>
> *The paper now presented records the results of such a detailed experiment. This experiment was practically confined to a small plant group, and is now, after eight years' pursuit, concluded in all essentials. Whether the plan upon which the separate experiments were conducted and carried out was the best suited to attain the desired end is left to the friendly decision of the reader.[2]*

[1,2]Peters, J. A., ed. 1959. *Classic Papers in Genetics.* Prentice-Hall, Englewood Cliffs, NJ.

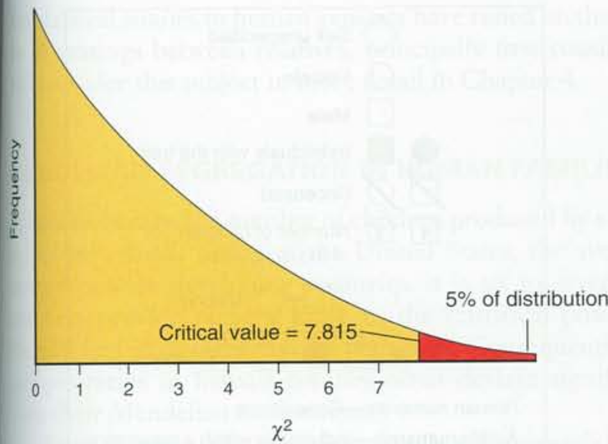


Figure 3.12 Distribution of a χ^2 statistic.

replications of the experiment to get it. The critical value is the point that cuts off the upper 5 percent of the distribution. By chance alone, the χ^2 statistic will exceed this value 5 percent of the time. Thus, if we perform an experiment once, compute a χ^2 statistic, and find that the statistic is greater than the critical value, we have either observed a rather unlikely set of results—something that happens less than 5 percent of the time—or there is a problem with the way the experiment was executed or with the appropriateness of the hypothesis. Assuming that the experiment was done properly, we are inclined to reject the hypothesis. Of course, we must realize that with this procedure we will reject a true hypothesis 5 percent of the time.

Thus, as long as we know the critical value, the χ^2 testing procedure leads us to a decision about the fate of the hypothesis. However, this critical value—and the shape of the associated frequency distribution—depend on the number of phenotypic classes in the experiment. Statisticians have tabulated critical values according to the **degrees of freedom** associated with the χ^2 statistic (**TABLE 3.2**). This index to the set of χ^2 distributions is determined by subtracting one from the number of phenotypic classes. In each of our examples, there are $4 - 1 = 3$ degrees of freedom. The critical value for the χ^2 distribution with 3 degrees of freedom is 7.815. For Mendel's data, the calculated χ^2 statistic is 0.51, much less than the critical value and

TABLE 3.2

Table of Chi-Square (χ^2) 5% Critical Values[a]

Degress of Freedom	5% Critical Value
1	3.841
2	5.991
3	7.815
4	9.488
5	11.070
6	12.592
7	14.067
8	15.507
9	16.919
10	18.307
15	24.996
20	31.410
25	37.652
30	43.773

[a]Selected entries from R. A. Fisher and Yates, 1943, *Statistical Table for Biological, Agricultural, and Medical Research*. Oliver and Boyd, London.

therefore no threat to the hypothesis being tested. However, for DeVries's data the calculated χ^2 statistic is 22.94, very much greater than the critical value. Thus, the observed data do not fit with the genetic hypothesis. Ironically, when DeVries presented these data in 1905, he judged them to be consistent with the genetic hypothesis. Unfortunately, he did not perform a χ^2 test. DeVries also argued that his data provided further evidence for the correctness and widespread applicability of Mendel's ideas—not the only time that a scientist has come to the right conclusion for the wrong reason.

KEY POINTS

- The chi-square statistic is calculated as $\chi^2 = \Sigma$ (observed number − expected number)^2/expected number, with the sum computed over all categories comprising the data.
- Each chi-square statistic is associated with an index, the degrees of freedom, which is equal to the number of data categories minus one.

Mendelian Principles in Human Genetics

Mendel's principles can be applied to study the inheritance of traits in humans.

The application of Mendelian principles to human genetics began soon after the rediscovery of Mendel's paper in 1900. However, because it is not possible to make controlled crosses with human beings, progress was obviously slow. The analysis of human heredity depends on family records, which are often incomplete. In addition, human beings—unlike experimental organisms—do not produce many progeny, making it difficult to discern Mendelian ratios, and humans are not maintained and observed in a controlled environment. For these and other reasons, human genetic analysis has been a difficult endeavor. Nonetheless, the drive to understand human heredity has been very strong, and today, despite all the obstacles, we have learned about thousands of human genes. **TABLE 3.3** lists some of the conditions they control. We discuss many of these conditions in later chapters of this book.

► **TABLE 3.3**

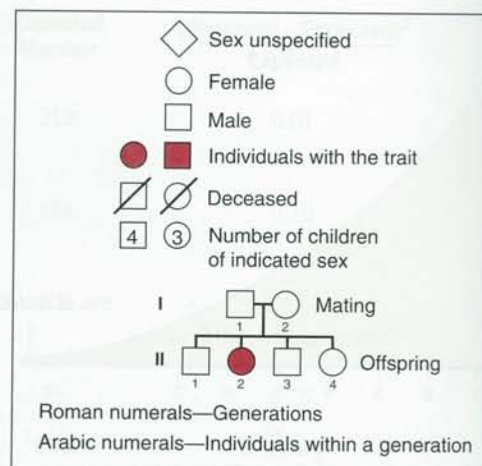
Inherited Condition in Human Beings

Dominant Traits
Achondroplasia (dwarfism)
Brachydactyly (short fingers)
Congenital night blindness
Ehler-Danlos syndrome (a connective tissue disorder)
Huntington's disease (a neurological disorder)
Marfan syndrome (tall, gangly stature)
Neurofibromatosis (tumorlike growths on the body)
Phenylthiocarbamide (PTC) tasting
Widow's peak
Woolly hair
Recessive Traits
Albinism (lack of pigment)
Alkaptonuria (a disorder of amino acid metabolism)
Ataxia telangiectasia (a neurological disorder)
Cystic fibrosis (a respiratory disorder)
Duchenne muscular dystrophy
Galactosemia (a disorder of carbohydrate metabolism)
Glycogen storage disease
Phenylketonuria (a disorder of amino acid metabolism)
Sickle-cell anemia (a hemoglobin disorder)
Tay-Sachs disease (a lipid storage disorder)

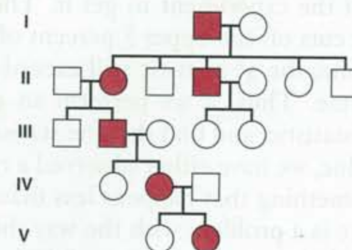
PEDIGREES

Pedigrees are diagrams that show the relationships among the members of a family (**FIGURE 3.13a**). It is customary to represent males as squares and females as circles. A horizontal line connecting a circle and a square represents a mating. The offspring of the mating are shown beneath the mates, starting with the first born at the left and proceeding through the birth order to the right. Individuals that have a genetic condition are indicated by coloring or shading. The generations in a pedigree are usually denoted by Roman numerals, and particular individuals within a generation are referred to by Arabic numerals following the Roman numeral.

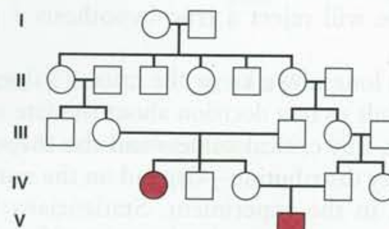
Traits caused by dominant alleles are the easiest to identify. Usually, every individual who carries the dominant allele manifests the trait, making it possible to trace the transmission of the dominant allele through the pedigree (**FIGURE 3.13b**). Every affected individual is expected to have at least one affected parent, unless, of course, the dominant allele has just appeared in the family as a result of a new mutation—a change in the gene itself. However, the frequency of most new mutations is very low—on the order of one in a million; consequently, the spontaneous appearance of a dominant condition is an extremely rare event. Dominant traits that are associated with reduced viability or fertility never become frequent in a population. Thus, most of the people who show such traits are heterozygous for the dominant allele. If their spouses do not have the trait, half their children should inherit the condition.



(a) Pedigree conventions



(b) Dominant trait



(c) Recessive trait

Figure 3.13 ► Mendelian inheritance in human pedigress. (*a*) Pedigree conventions. (*b*) Inheritance of a dominant trait. The trait appears in each generation. (*c*) Inheritance of a recessive trait. The two affected individuals are the offspring of relatives.

Recessive traits are not so easy to identify because they may occur in individuals whose parents are not affected. Sometimes several generations of pedigree data are needed to trace the transmission of a recessive allele (**FIGURE 3.13c**). Nevertheless, a large number of recessive traits have been observed in human beings—at last count, over 4000. Rare recessive traits are more likely to appear in a pedigree when spouses are related to each other—for example, when they are first cousins. This increased incidence occurs because relatives share alleles by virtue of their common ancestry. Siblings share one-half their alleles, half siblings one-fourth their alleles, and first cousins one-eighth their alleles. Thus, when such relatives mate, they have a greater chance of producing a child who is homozygous for a particular recessive allele than do unrelated parents. Many of

the classical studies in human genetics have relied on the analysis of matings between relatives, principally first cousins. We will consider this subject in more detail in Chapter 4.

MENDELIAN SEGREGATION IN HUMAN FAMILIES

In human beings, the number of children produced by a couple is typically small. Today in the United States, the average is around two. In developing countries, it is six to seven. Such numbers provide nothing close to the statistical power that Mendel had in his experiments with peas. Consequently, phenotypic ratios in human families often deviate significantly from their Mendelian expectations.

As an example, let's consider a couple who are each heterozygous for a recessive allele that, in homozygous condition, causes cystic fibrosis, a serious disease in which breathing is impaired by an accumulation of mucus in the lungs and respiratory tract. If the couple were to have four children, would we expect exactly three to be unaffected and one to be affected by cystic fibrosis? The answer is no. Although this is a possible outcome, it is not the only one. There are, in fact, five distinct possibilities:

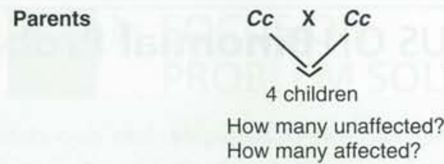
1. Four unaffected, none affected.
2. Three unaffected, one affected.
3. Two unaffected, two affected.
4. One unaffected, three affected.
5. None unaffected, four affected.

Intuitively, the second outcome seems to be the most likely, since it conforms to Mendel's 3:1 ratio. We can calculate the probability of this outcome, and of each of the others, by using Mendel's principles and by treating each birth as an independent event (**FIGURE 3.14**).

For a particular birth, the chance that the child will be unaffected is 3/4. The probability that all four children will be unaffected is therefore $(3/4) \times (3/4) \times (3/4) \times (3/4) = (3/4)^4 = 81/256$. Similarly, the chance that a particular child will be affected is 1/4; thus, the probability that all four will be affected is $(1/4)^4 = 1/256$. To find the probabilities for the three other outcomes, we need to recognize that each actually represents a collection of distinct events. The outcome of three unaffected children and one affected child, for instance, comprises four distinct events; if we let U symbolize an unaffected child and A an affected child, and if we write the children in their order of birth, we can represent these events as

UUUA, UUAU, UAUU, and AUUU

Because each has probability $(3/4)^3 \times (1/4)$, the total probability for three unaffected children and one affected, regardless of birth order, is $4 \times (3/4)^3 \times (1/4)$. The coefficient 4 is the number of ways in which three children could be unaffected and one could be affected in a family with four children. Similarly, the probability for two unaffected children and two affected is $6 \times (3/4)^2 \times (1/4)^2$, since in this case there are six distinct events. The probability for one unaffected child and three affected is $4 \times (3/4) \times (1/4)^3$, since



Number of children that are:

Unaffected	Affected	Probability
4	0	1 x (3/4) x (3/4) x (3/4) x (3/4) = 81/256
3	1	4 x (3/4) x (3/4) x (3/4) x (1/4) = 108/256
2	2	6 x (3/4) x (3/4) x (1/4) x (1/4) = 54/256
1	3	4 x (3/4) x (1/4) x (1/4) x (1/4) = 12/256
0	4	1 x (1/4) x (1/4) x (1/4) x (1/4) = 1/256

Probability distribution:

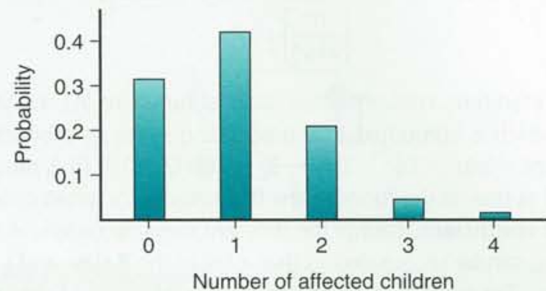


Figure 3.14 ► Probability distribution for families with four children segregating a recessive trait.

in this case there are four distinct events. **FIGURE 3.14** summarizes the calculations in the form of a probability distribution. As anticipated, three unaffected children and one affected child is the most probable outcome (probability 108/256).

In this example, the children fall into two possible phenotypic classes. Because there are only two classes, the probabilities associated with the various outcomes are called **binomial probabilities.** The Focus on Binomial Probabilities generalizes the analysis of this example to other situations involving two phenotypic classes.

GENETIC COUNSELING

The diagnosis of genetic conditions is often a difficult process. Typically, diagnoses are made by physicians who have been trained in genetics. The study of these conditions requires a great deal of careful research, including examining patients, interviewing relatives, and sifting through vital statistics on births, deaths, and marriages. The accumulated data provide the basis for defining the condition clinically and for determining its mode of inheritance.

Prospective parents may want to know whether their children are at risk to inherit a particular condition, especially if other family members have been affected. It is the responsibility of the genetic counselor to assess such risks and to explain them to the prospective parents. Risk assessment requires familiarity with probability and statistics, as well as a thorough knowledge of genetics.

As an example, let's consider a pedigree showing the inheritance of **nonpolypoid colorectal cancer** (**FIGURE 3.15**). This

▸ FOCUS ON Binomial Probabilities

The progeny of crosses sometimes segregate into two distinct classes—for example, male or female, healthy or diseased, normal or mutant, dominant phenotype or recessive phenotype. To be general, we can refer to these two kinds of progeny as P and Q, and note that for any individual offspring, the probability of being P is p and the probability of being Q is q. Because there are only two classes, $q = 1 - p$. Suppose that the total number of progeny is n and that each one is produced independently. We can calculate the **binomial probability** that exactly x of the progeny will fall into one class and y into the other:

$$\text{Probability of } x \text{ in class P and } y \text{ in class Q} = \left[\frac{n!}{x!\,y!}\right] p^x q^y$$

The bracketed term contains three factorial functions ($n!$, $x!$, and $y!$), each of which is computed as a descending series of products. For example, $n! = n(n-1)(n-2)(n-3) \ldots (3)(2)(1)$. If 0! is needed, it is defined as one. In the formula, the bracketed term, often called the **binomial coefficient,** counts the different ways, or orders, in which n offspring can be segregated so that x fall in the P class and y fall in the Q class. The other term, $p^x q^y$, gives the probability of obtaining a particular way or order. Because each of the orders is equally likely, multiplying this term by the bracketed term gives the probability of obtaining x progeny in the P class and y in the Q class, regardless of the order of occurrence.

If, for fixed values of n, p, and q, we systematically vary x and y, we can calculate a whole set of probabilities. This set constitutes a binomial probability distribution. With the distribution, we can answer questions such as "What is the probability that x will exceed a particular value?" or "What is the probability that x will lie between two particular values?" For example, let's consider a family with six children. What is the probability that at least four will be girls? To answer this question, we note that for any given child, the probability that it will be a girl (p) is 1/2 and the probability that it will be a boy (q) is also 1/2. The probability that exactly four children in a family will be girls (and two will be boys) is therefore $[(6!)/(4!\,2!)](1/2)^4\,(1/2)^2 = 15/64$, which is one of the terms in the binomial distribution. However, the probability that at least four will be girls (and that no more than two will be boys) is the sum of three terms from this distribution:

Event	Binomial Formula	Probability
4 girls and 2 boys	$[(6!)/(4!\,2!)] \times (1/2)^4\,(1/2)^2 =$	15/64
5 girls and 1 boy	$[(6!)/(5!\,1!)] \times (1/2)^5\,(1/2)^1 =$	6/64
6 girls and 0 boys	$[(6!)/(6!\,0!)] \times (1/2)^6\,(1/2)^0 =$	1/64

Therefore, the answer is $(15/64) + (6/64) + (1/64) = 22/64$.

The binomial distribution also provides answers to other kinds of questions. For example, what is the probability that at least one but no more than four of the children will be girls? Here the answer is the sum of four terms:

Event	Binomial Formula	Probability
1 girl and 5 boys	$[(6!)/(1!\,5!)] \times 6/64$	$(1/2)^1\,(1/2)^5 =$
2 girls and 4 boys	$[(6!)/(2!\,4!)] \times 15/64$	$(1/2)^2\,(1/2)^4 =$
3 girls and 3 boys	$[(6!)/(3!\,3!)] \times 20/64$	$(1/2)^3\,(1/2)^3 =$
4 girls and 2 boys	$[(6!)/(4!\,2!)] \times 15/64$	$(1/2)^4\,(1/2)^2 =$

Summing up, we find that the answer is 56/64.

Let's now consider the example discussed in the section on Mendelian Segregation in Human Families. A man and a woman, who are both heterozygous for the recessive mutant allele that causes cystic fibrosis, plan to have four children. What is the chance that one of these children will have cystic fibrosis and the other three will not? We have already seen by enumeration that the answer to this question is 108/256 (see **FIGURE 3.14**). However, this answer could also be obtained by using the binomial formula. The probability that a particular child will be affected is $p = 1/4$, and the probability that it will not be affected is $q = 3/4$. The total number of children is $n = 4$, the number of affected children is $x = 1$, and the number of unaffected children is $y = 3$. Putting all this together, we can calculate the probability that exactly one of the couple's four children will have cystic fibrosis as

$$[4!/(1!3!)]\,(1/4)^1\,(3/4)^3 = 4 \times (1/4) \times (27/64) = 108/256$$

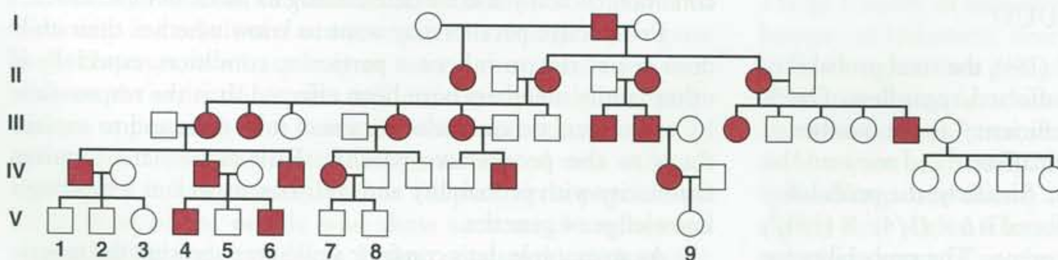


Figure 3.15 ▸ Pedigree showing the inheritance of hereditary non-polypoid colorectal cancer.

disease is one of several types of cancer that are inherited. It is due to a dominant mutation that affects about 1 in 500 individuals in the general population. The median age when hereditary nonpolypoid colorectal cancer appears in an individual who carries the mutation is 42. In the pedigree, we see that the cancer is manifested in at least one individual in each generation and that every affected individual has an affected parent. These facts are consistent with the dominant mode of inheritance of this disease.

The counseling issue arises in generation V. Among the nine individuals shown, two are affected and seven are not. Yet each of the seven unaffected individuals had one affected parent who must have been heterozygous for the cancer-causing mutation. Some of these seven unaffected individuals may therefore have inherited the mutation and would be at risk to develop nonpolypoid colorectal cancer later in life. Only time will tell. As the unaffected individuals age, those who carry the mutation will be at increased risk to develop the disease. Thus, the longer they remain unaffected, the greater the probability that they are actually not carriers. In this situation, the risk is a function of an individual's age and must be ascertained empirically from data on the age of onset of the disease among individuals from the same population, if possible from the same family. Each of the seven unaffected individuals will, of course, have to live with the anxiety of being a possible carrier of the cancer-causing mutation. Furthermore, at some point they will have to decide if they wish to reproduce and risk transmitting the mutation to their children. We shall discuss other inherited cancers and related counseling issues in Chapter 22.

As another example, consider the situation shown in **FIGURE 3.16**. A couple, denoted R and S in Figure 3.16a, is concerned about the possibility that they will have a child (T) with **albinism,** a recessive condition characterized by a complete

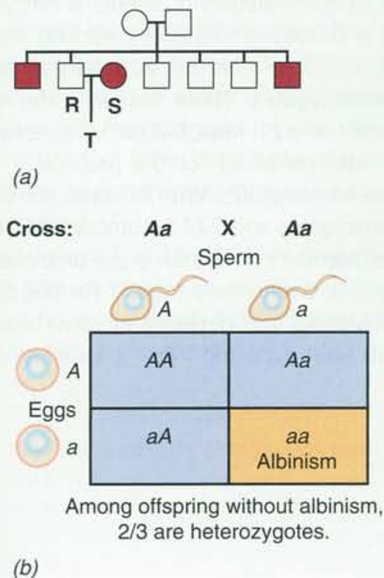


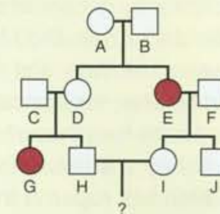
Figure 3.16 ► Genetic counseling in a family with albinism. (*a*) Pedigree showing the inheritance of albinism. (*b*) Punnett square showing that, among offspring without albinism, the frequency of heterozygotes is 2/3.

► FOCUS ON PROBLEM SOLVING

Making Predictions from Pedigrees

THE PROBLEM

This pedigree shows the inheritance of a recessive trait in humans. Individuals that have the trait are homozygous for a recessive allele *a*. If H and I, who happen to be first cousins, marry and have a child, what is the chance that this child will have the recessive trait?



FACTS AND CONCEPTS

1. The child can show a recessive trait only if both of its parents carry the recessive allele.

2. One parent (H) has a sister (G) with the trait.

3. The other parent (I) has a mother (E) with the trait.

4. The chance that a heterozygote will transmit a recessive allele to its offspring is 1/2.

5. In a mating between two heterozygotes, 2/3 of the offspring that do not show the trait are expected to be heterozygotes (see **FIGURE 3.16b**).

ANALYSIS AND SOLUTION

I must be a heterozygous carrier of the recessive allele because her mother E is homozygous for it but she herself does not show the trait. I therefore has a 1/2 chance of transmitting the recessive allele to her child. Because H's sister has the trait, both of her parents must be heterozygotes. H, who does not show the trait, therefore has a 2/3 chance of being a heterozygote, and if he is, there is a 1/2 chance that he will transmit the recessive allele to his child. Putting all these factors together, we calculate the chance that the child of H and I will show the trait as 1/2 (the chance that I transmits the recessive allele) × 2/3 (the chance that H is a heterozygote) × 1/2 (the chance that H transmits the recessive allele assuming that he is a heterozygote) = 1/6, which is a fairly substantial risk.

For further discussion go to your *WileyPLUS* course.

absence of melanin pigment in the skin, eyes, and hair. S, the prospective mother, has albinism, and R, the prospective father, has two siblings with albinism. It would therefore seem that the child has some risk of being born with albinism.

This risk depends on two factors: (1) the probability that R is a heterozygous carrier of the albinism allele (*a*), and (2) the probability that he will transmit this allele to T if he actually is a carrier. S, who is obviously homozygous for the albinism allele, must transmit this allele to her offspring.

To determine the first probability, we need to consider the possible genotypes for R. One of these, that he is homozygous for the recessive allele (*aa*), is excluded because we know that he does not have albinism himself. However, the other two genotypes, *AA* and *Aa*, remain distinct possibilities. To calculate the probabilities associated with each of these, we note that both of R's parents must be heterozygotes, because they have had two children with albinism. The mating that produced R was therefore $Aa \times Aa$, and from such a mating we would expect 2/3 of the offspring without albinism to be *Aa* and 1/3 to be *AA* (**FIGURE 3.16b**). Thus, the probability that R is a heterozygous carrier of the albinism allele is 2/3. To determine the probability that he will transmit this allele to his child, we simply note that *a* will be present in half of his gametes.

In summary, the risk that T will be *aa*

= [Probability that R is *Aa*] × [Probability that R transmits *a*, assuming that R is *Aa*]

$= (2/3) \times (1/2) = (1/3)$

The example in Figure 3.16 illustrates a simple counseling situation in which the risk can be determined precisely. Often the circumstances are much more complicated, making the task of risk assessment quite difficult. The genetic counselor's responsibility is to analyze the pedigree information and determine the risk as precisely as possible.

For practice in calculating genetic risks, work through the example in the Focus on Problem Solving.

KEY POINTS

- Pedigrees are used to identify dominant and recessive traits in human families.
- The analysis of pedigrees allows genetic counselors to assess the risk that an individual will inherit a particular trait.

Basic Exercises

ILLUSTRATE BASIC GENETIC ANALYSIS

1. Two highly inbred strains of mice, one with black fur and the other with gray fur, were crossed, and all of the offspring had black fur. Predict the outcome of intercrossing the offspring.

Answer: The two strains of mice are evidently homozygous for different alleles of a gene that controls fur color: *G* for black fur and *g* for gray fur; the *G* allele is dominant because all the F_1 animals are black. When these mice, genotypically *Gg*, are intercrossed, the *G* and *g* alleles will segregate from each other to produce an F_2 population consisting of three genotypes, *GG*, *Gg*, and *gg*, in the ratio 1:2:1. However, because of the dominance of the *G* allele, the *GG* and *Gg* genotypes will have the same phenotype (black fur); thus, the phenotypic ratio in the F_2 will be 3 black:1 gray.

2. A plant heterozygous for three independently assorting genes, *Aa Bb Cc*, is self-fertilized. Among the offspring, predict the frequency of (a) *AA BB CC* individuals, (b) *aa bb cc* individuals, (c) individuals that are either *AA BB CC* or *aa bb cc*, (d) *Aa Bb Cc* individuals, (e) individuals that are not heterozygous for all three genes.

Answer: Because the genes assort independently, we can analyze them one at a time to obtain the answers to each of the questions. (a) When *Aa* individuals are selfed, 1/4 of the offspring will be *AA*; likewise, for the *B* and *C* genes, 1/4 of the individuals will be *BB* and 1/4 will be *CC*. Thus, we can calculate the frequency (that is, the probability) of *AA BB CC* offspring as $(1/4) \times (1/4) \times (1/4) = 1/64$. (b) The frequency of *aa bb cc* individuals can be obtained using similar reasoning. For each gene the frequency of recessive homozygotes among the offspring is 1/4. Thus, the frequency of triple recessive homozygotes is $(1/4) \times (1/4) \times (1/4) = 1/64$. (c) To obtain the frequency of offspring that are either triple dominant homozygotes or triple recessive homozygotes—these are mutually exclusive events—we sum the results of (a) and (b): $1/64 + 1/64 = 2/64 = 1/32$. (d) To obtain the frequency of offspring that are triple heterozygotes, again we multiply probabilities. For each gene, the frequency of heterozygous offspring is 1/2; thus, the frequency of triple heterozygotes should be $(1/2) \times (1/2) \times (1/2) = 1/8$. (e) Offspring that are not heterozygous for all three genes occur with a frequency that is one minus the frequency calculated in (d). Thus, the answer is $1 - 1/8 = 7/8$.

3. Two true-breeding strains of peas, one with tall vines and violet flowers and the other with dwarf vines and white flowers, were crossed. All the F_1 plants were tall and produced violet flowers. When these plants were backcrossed to the dwarf, white parent strain, the following offspring were obtained: 53 tall, violet; 48 tall, white; 47 dwarf, violet; 52 dwarf, white. Do the genes that control vine length and flower color assort independently?

Answer: The hypothesis of independent assortment of the vine length and flower color genes must be evaluated by calculating a chi-square test statistic from the experimental results. To obtain this statistic, the results must be compared to the predictions of the genetic hypothesis. Under the assumption that the two genes assort independently, the four phenotypic classes in the F_2 should each be 25 percent of the total (200); that is, each should contain 50 individuals. To compute the chi-square statistic, we must obtain the difference between each observation and its predicted value, square these differences, divide each squared difference by the predicted value, and then sum the results:

$$\chi^2 = (53 - 50)^2/50 + (48 - 50)^2/50 + (47 - 50)^2/50 + (52 - 50)^2/50 = 0.52$$

Chapter 24
Population Genetics

CHAPTER OUTLINE

- The Theory of Allele Frequencies
- Natural Selection
- Random Genetic Drift
- Populations in Genetic Equilibrium

Pitcairn Island in the south Pacific.

A Remote Colony

In September 1787, Lieutenant William Bligh and a crew of 45 men set sail from England aboard the ship H.M.S. *Bounty*. Their destination was the Pacific island of Tahiti, where they were to collect breadfruit tree saplings for transplantation to the Caribbean island of Jamaica. Because their passage around Cape Horn was blocked by ferociously bad weather, they sailed to Tahiti by crossing the south Atlantic, rounding the Cape of Good Hope, and then traversing the southern Indian Ocean and the western Pacific. Their voyage was long and difficult. When they finally reached Tahiti, they relaxed there and enjoyed the hospitality of the local people. After collecting the breadfruit saplings, Bligh and his crew departed Tahiti on April 6, 1789, bound for the Caribbean. Barely three weeks into the voyage, the crew mutinied. Led by Bligh's friend and chief subordinate Fletcher Christian, the mutineers put Bligh and his supporters into the ship's launch and set them adrift in the lonely waters of the south Pacific. Eventually Bligh and his men reached civilization. The mutineers initially returned to Tahiti, where some decided to stay, but nine of them, including Fletcher Christian, resolved to find another place to live. Along with a group of Polynesians—six men, twelve women, and a baby—they set sail in the *Bounty*, and on January 15, 1790, landed on Pitcairn Island, an uninhabited speck of land 1350 miles from Tahiti. Pitcairn Island had been discovered decades earlier, but because cartographers had put it in the wrong place on their charts, it held promise as a refuge for the mutineers. On January 23, 1790, Fletcher Christian and his followers burned the *Bounty* and set about establishing their new home.

Life on Pitcairn Island was not easy. The men fought over land and women, and the women murdered some of the men. In 1808, the island was visited by an American whaling ship, which found that only one of the original mutineers was still alive. British ships subsequently stopped at the island, and in 1838, Pitcairn Island was formally incorporated into the British Empire. By 1855 the population of the colony had increased to nearly 200, which was more than it could sustain, and in 1856 all the people were moved to Norfolk Island, a former British penal colony 3500 miles away. Two years later, 17 of the former inhabitants returned to Pitcairn Island to reestablish the colony, which has survived for nearly 150 years and today is home to about 50 people, all descendants of the original settlers.

The population on Pitcairn Island is the result of mixing two different groups of people, Britons and Polynesians. The offspring of the original settlers received genes from each of these groups, and when they reproduced, some of these genes were transmitted to their offspring and ultimately to the current members of the population. Which of the founding genes were passed down through time? How did factors such as the health, vigor, and reproductive ability of the people, and the ways in which they chose mates, influence the pathways of genetic descent? Did any of the genes mutate as they were transmitted through time? How did migration to and from the island affect its genetic composition? Has the island's genetic diversity increased, decreased, or remained the same? What is the significance of the population's size? Has the genetic composition of the population changed over time—that is, has it evolved?

These and other questions about the genetic makeup and history of the people on Pitcairn Island fall within the purview of *population genetics*, a discipline that studies genes in groups of individuals. Population genetics examines allelic variation among individuals, the transmission of allelic variants from parents to offspring generation after generation, and the temporal changes that occur in the genetic makeup of a population because of systematic and random evolutionary forces. In this chapter, we shall investigate how these forces—mutation, migration, selection, and random genetic drift—shape the genetic composition of a population. We begin with an introduction to the basic methods of population genetic analysis. As we shall see, these methods focus on the frequencies of the alleles that are present in the members of the population.

The Theory of Allele Frequencies

When the members of a population mate randomly, it is easy to predict the frequencies of the genotypes from the frequencies of their constituent alleles.

The theory of population genetics is a theory of allele frequencies. Each gene in the genome exists in different allelic states, and, if we focus on a particular gene, a diploid individual is either a homozygote or a heterozygote. Within a population of individuals, we can calculate the frequencies of the different types of homozygotes and heterozygotes of a gene, and from these frequencies we can estimate the frequency of each of the gene's alleles. These calculations are the foundation for population genetics theory.

ESTIMATING ALLELE FREQUENCIES

Because an entire population is usually too large to study, we resort to analyzing a representative sample of individuals from it. **TABLE 24.1** presents data from a sample of people who were tested for the M-N blood types. These blood types are determined by two alleles of a gene on chromosome 4: L^M, which produces the M blood type, and L^N, which produces the N blood type (see Chapter 4). People who are L^ML^N heterozygotes have the MN blood type.

▶ TABLE 24.1

Frequency of the M-N Blood Types in a Sample of 6129 Individuals

Blood Type	Genotype	Number of Individuals
M	L^ML^M	1787
MN	L^ML^N	3039
N	L^NL^N	1303

To estimate the frequencies of the L^M and L^N alleles, we simply calculate the incidence of each allele among all the alleles sampled:

1. Because each individual in the sample carries two alleles of the blood-type locus, the total number of alleles in the sample is two times the sample size: $2 \times 6129 = 12{,}258$.
2. The frequency of the L^M allele is two times the number of L^ML^M homozygotes plus the number of L^ML^N heterozygotes, all divided by the total number of alleles sampled: $[(2 \times 1787) + 3039]/12{,}258 = 0.5395$.
3. The frequency of the L^N allele is two times the number of L^NL^N homozygotes plus the number of L^ML^N heterozygotes, all divided by the total number of alleles sampled: $[(2 \times 1303) + 3039]/12{,}258 = 0.4605$.

Thus, letting p represent the frequency of the L^M allele and letting q represent the frequency of the L^N allele, we estimate that in the population from which the sample was taken, $p = 0.5395$ and $q = 0.4605$. Furthermore, because L^M and L^N represent 100 percent of the alleles of this particular gene, $p + q = 1$.

When directly counting the number of alleles in a sample is not possible because one of the alleles is dominant, we cannot use this method to estimate the allele frequencies. However, another method, discussed in a later section, does provide these estimates.

When the gene under study is X-linked, we only need to count the different alleles in males. For example, in a sample of 200 men, 24 have X-linked color blindness and all the others have normal color vision. Assuming that each color-blind man is hemizygous for the same mutant allele, we estimate the fre-

quency of that allele to be $24/200 = 0.12$ and the frequency of the normal allele to be $1 - 0.12 = 0.88$.

In these examples, each of the alleles has a reasonably high frequency—one that can be estimated reliably with a sample of moderate size. However, some alleles have frequencies of 0.01 or less, and estimating their frequencies, or even detecting them, requires a large sample. Whenever the second most frequent allele of a gene has a frequency greater than 0.01, we refer to the situation as a genetic **polymorphism.** Later in this chapter we shall discuss the evolutionary forces that maintain genetic polymorphisms in nature.

RELATING GENOTYPE FREQUENCIES TO ALLELE FREQUENCIES: THE HARDY–WEINBERG PRINCIPLE

Do the estimated allele frequencies have any predictive power? Can we use them to predict the frequencies of genotypes? In the first decade of the twentieth century, these questions were posed independently by G. H. Hardy, a British mathematician, and by Wilhelm Weinberg, a German physician. In 1908 Hardy and Weinberg each published papers describing a mathematical relationship between allele frequencies and genotype frequencies. This relationship, now called the **Hardy–Weinberg principle,** allows us to predict a population's genotype frequencies from its allele frequencies.

Let's suppose that in a population a particular gene is segregating two alleles, A and a, and that the frequency of A is p and that of a is q. If we assume that the members of the population mate randomly, then the diploid genotypes of the next generation will be formed by the random union of haploid eggs and haploid sperm (**FIGURE 24.1**). The probability that an egg (or sperm) carries A is p, and the probability that it carries a is q. Thus, the probability of producing an AA homozygote in the population is simply $p \times p = p^2$, and the probability of producing an aa homozygote is $q \times q = q^2$. For the Aa heterozygotes, there are two possibilities: An A sperm can unite with an a egg, or an a sperm can unite with an A egg. Each of these events occurs with probability $p \times q$, and because they are equally likely, the total probability of forming an Aa zygote is $2pq$. Thus, on the assumption of random mating, the predicted frequencies of the three genotypes in the population are:

Genotype	Frequency
AA	p^2
Aa	$2pq$
aa	q^2

These predicted frequencies can be obtained by expanding the binomial expression $(p + q)^2 = p^2 + 2pq + q^2$. Population geneticists refer to them as the Hardy–Weinberg genotype frequencies.

The key assumption underlying the Hardy–Weinberg principle is that the members of the population mate at random with respect to the gene under study. This assumption means that the adults of the population essentially form a pool of gametes that, at fertilization, combine randomly to produce the zygotes of the next generation. If these zygotes have equal chances of surviving to the adult stage, then the genotype frequencies created at the time of fertilization will be preserved, and when the next generation reproduces, these frequencies will once again appear in the offspring. Thus, with random mating and no differential survival or reproduction among the members of the population, the Hardy–Weinberg genotype frequencies—and, of course, the underlying allele frequencies—persist generation after generation. This condition is referred to as the *Hardy–Weinberg equilibrium.* Later in this chapter we shall consider forces that upset this equilibrium by altering allele frequencies; these forces—mutation, migration, natural selection, and random genetic drift—play key roles in the evolutionary process.

Figure 24.1 ▸ Punnett square showing the Hardy–Weinberg principle.

APPLICATIONS OF THE HARDY–WEINBERG PRINCIPLE

The intellectual roots of the Hardy–Weinberg principle are discussed in A Milestone in Genetics later in this chapter. Here, let's return to the M-N blood type example to see how the Hardy–Weinberg principle applies to a real population. From the sample data given in **TABLE 24.1**, the frequency of the L^M allele was estimated to be $p = 0.5395$ and the frequency of the L^N allele was estimated to be $q = 0.4605$. With the Hardy–Weinberg principle, we can now use these frequencies to predict the genotype frequencies of the M-N blood type gene:

Genotype	Hardy–Weinberg Frequency
L^ML^M	$p^2 = (0.5395)^2 = 0.2911$
L^ML^N	$2pq = 2\ (0.5395)\ (0.4605) = 0.4968$
L^NL^N	$q^2 = (0.4605)^2 = 0.2121$

Do these predictions fit with the original data from which the two allele frequencies were estimated? To answer this question, we must compare the observed genotype numbers with numbers predicted by the Hardy–Weinberg principle. We obtain these predicted numbers by multiplying the Hardy–Weinberg frequencies by the size of the sample taken from the population:

Genotype	Predicted Number
L^ML^M	$0.2911 \times 6129 = 1784.2$
L^ML^N	$0.4968 \times 6129 = 3044.8$
L^NL^N	$0.2121 \times 6129 = 1300.0$

The results are extraordinarily close to the original sample data presented in **TABLE 24.1**. We can check for agreement between the observed and predicted numbers by calculating a chi-square statistic (see Chapter 3):

$$\chi^2 = \frac{(1787 - 1784.2)^2}{1784.2} + \frac{(3039 - 3044.8)^2}{3044.8} + \frac{(1303 - 1300.0)^2}{1300.0}$$

$$= 0.223$$

This chi-square statistic has $3 - 2 = 1$ degree of freedom because (1) the sum of the three predicted numbers is fixed by the sample size, and because (2) the allele frequency p was estimated directly from the sample data. (The frequency q can be estimated indirectly as $1 - p$ and therefore does not reduce the degrees of freedom any further.) The critical value for a chi-square statistic with one degree of freedom is 3.841 (see **TABLE 3.2**), which is much greater than the observed value. Consequently, we conclude that the predicted genotype frequencies are in agreement with the observed frequencies in the sample, and furthermore, we infer that in the population from which the sample was obtained, the M-N genotypes are in Hardy–Weinberg proportions—a finding that is not too surprising given that marriage is usually not based on blood type.

The preceding analysis indicates how we can use the Hardy–Weinberg principle to predict genotype frequencies from allele frequencies. Can we turn the Hardy–Weinberg principle around and use it to predict allele frequencies from genotype frequencies? For example, in the United States, the incidence of the recessive metabolic disorder phenylketonuria (PKU) is about 0.0001. Does this statistic allow us to calculate the frequency of the mutant allele that causes PKU?

We cannot proceed as before by counting the different types of alleles, mutant and normal, that are present in the population because heterozygotes and normal homozygotes are phenotypically indistinguishable. Instead, we must proceed by applying the Hardy–Weinberg principle in reverse to estimate the mutant allele frequency. The incidence of PKU, 0.0001, represents the frequency of mutant homozygotes in the population. Under the assumption of random mating, these individuals should occur with a frequency equal to the square of the mutant allele frequency. Denoting this allele frequency by q, we have

$$q^2 = 0.0001$$
$$q = \sqrt{0.0001} = 0.01$$

Thus, 1 percent of the alleles in the population are estimated to be mutant. Using the Hardy–Weinberg principle in the usual way, we can then predict the frequency of people in the population who are heterozygous carriers of the mutant allele:

$$\text{Carrier frequency} = 2pq = 2(0.99)(0.01) = 0.0198$$

Thus, approximately 2 percent of the population are predic to be carriers.

The Hardy–Weinberg principle also applies to X-lin genes and to genes with multiple alleles. For an X-linked g such as the one that controls color vision, the allele frequen are estimated from the frequencies of the genotypes in males, the frequencies of the genotypes in females are obtained applying the Hardy–Weinberg principle to these estimated al frequencies. (We assume, of course, that the allele frequencies the same in the two sexes.) If the frequency of the allele for r mal color vision (C) is $p = 0.88$ (taken from our earlier exam and the frequency of the allele for color blindness (c) is $q = 0$ then, under the assumptions of random mating and equal al frequencies in the two sexes, we have:

Sex	Genotype	Frequency	Phenotype
Males	C	$p = 0.88$	Normal visio
	c	$q = 0.12$	Color blind
Females	CC	$p^2 = 0.77$	Normal visio
	Cc	$2pq = 0.21$	Normal visio
	cc	$q^2 = 0.02$	Color blind

For genes with multiple alleles, the Hardy–Weinberg ge type proportions are obtained by expanding a multinomial exp sion. For example, the A–B–O blood types are determined three alleles I^A, I^B, and i. If the frequencies of these are p, q, an respectively, then the frequencies of the six different genotype the A–B–O blood-typing system are obtained by expanding trinomial $(p + q + r) = p^2 + q^2 + r^2 + 2pq + 2qr + 2pr$:

Blood Type	Genotype	Frequency
A	I^AI^A	p^2
	I^Ai	$2pr$
B	I^BI^B	q^2
	I^Bi	$2qr$
AB	I^AI^B	$2pq$
O	ii	r^2

EXCEPTIONS TO THE HARDY–WEINBERG PRINCIPL

There are many reasons why the Hardy–Weinberg princi might not apply to a particular population. Mating might no random, the members of the population carrying different a les might not have equal chances of surviving and reproduci the population might be subdivided into partially isolated u or it might be an amalgam of different populations that h come together recently by migration. We now briefly consi each of these exceptions to the Hardy–Weinberg principle.

1. *Nonrandom mating*. Random mating is the key assumpt underlying the Hardy–Weinberg principle. If mating is random, the simple relationship between allele frequen and genotype frequencies breaks down. There are two w

n which the members of a population might mate nonran-lomly. First, they might mate with each other because they ıre genetically related—for example, because they are sib-ings or first cousins. We refer to this type of nonrandom nating as *consanguineous mating* (see Chapter 4). Second, the nembers of the population might mate with each other because they are phenotypically similar—for example, because the mates have the same stature or skin color. We efer to this type of nonrandom mating as *assortative mating*.

Consanguineous mating and assortative mating have the same qualitative effect; they reduce the frequency of hetero-zygotes and increase the frequency of homozygotes compared o the Hardy–Weinberg genotype frequencies. For the case of consanguineous mating, we can quantify this effect by ısing the inbreeding coefficient, F (see Chapter 4). Let's sup-pose that a gene has two alleles, A and a, with respective fre-quencies p and q, and that the population in which the gene is segregating has reached a level of inbreeding measured by F. (Recall from Chapter 4 that the range of F is between 0 and 1, with 0 corresponding to no inbreeding and 1 corresponding to complete inbreeding.) The genotype frequencies in this population are given by the following formulas:

Genotype	Frequency with Consanguineous Mating
AA	$p^2 + pqF$
Aa	$2pq - 2pqF$
aa	$q^2 + pqF$

From these formulas, it is clear that the frequencies of the two homozygotes have increased compared to the Hardy–Weinberg frequencies and that the frequency of the hetero-zygotes has decreased compared to the Hardy–Weinberg frequency. Notice that for each homozygote, the increase in frequency is exactly half the decrease in the frequency of the heterozygotes. Furthermore, each change in genotype fre-quency is directly proportional to the inbreeding coefficient. For a population that is completely inbred, $F = 1$, and the genotype frequencies become:

Genotype	Frequency with $F = 1$
AA	p
Aa	0
Aa	q

With assortative mating, mathematical expressions for the genotype frequencies are more complicated than those for consanguineous mating and are beyond the scope of this book. However, assortative mating has the same general effect as consanguineous mating: it increases the frequency of homozygotes and decreases the frequency of heterozy-gotes. These changes occur because phenotypically similar individuals tend to have similar genotypes. Thus, when such individuals mate, they tend to produce more homozygous offspring than do randomly mated individuals.

2. *Unequal survival*. If zygotes produced by random mating have different survival rates, we would not expect the geno-type frequencies of the individuals that develop from these zygotes to conform to the Hardy–Weinberg predictions. For example, consider a randomly mating population of *Drosophila* that is segregating two alleles, A_1 and A_2, of an autosomal gene. A sample of 200 adults from this popula-tion yielded the following data:

Genotype	Observed Number	Expected Number
A_1A_1	26	46.1
A_1A_2	140	99.8
A_2A_2	34	54.1

The expected numbers were obtained by estimating the frequencies of the two alleles among the flies in the sample; the frequency of the A_1 allele is $(2 \times 26 + 140)/(2 \times 200) = 0.48$, and the frequency of the A_2 allele is $1 - 0.48 = 0.52$. Then the Hardy–Weinberg formulas were applied to these estimated frequencies. Obviously, the expected numbers are not in agreement with the observed numbers, which show an excess of heterozygotes and a dearth of both types of homozygotes. Here the disagreement is so obvious that a chi-square calculation to test the goodness of fit between the observed and expected numbers is unnecessary. The ex-planation for the disagreement probably lies with differen-tial survival of the three genotypes during development from the zygote to the adult stage. The A_1A_2 heterozygotes survive better than either of the two homozygotes. Unequal survival rates can therefore lead to genotype frequencies that deviate from the Hardy–Weinberg predictions.

3. *Population subdivision*. When a population is a single inter-breeding unit, it is said to be **panmictic. Panmixis** (the noun) implies that any member of the population is able to mate with any other member—that is, there are no geo-graphical or ecological barriers to mating in the population. In nature, however, populations are often subdivided. We can think of fish living in a group of lakes that are intermit-tently connected by rivers, or of birds living on a chain of islands in an archipelago. Such populations are structured by geographical and ecological features that might be cor-related with genetic differences. For example, the fish in one lake might have a high frequency of allele A, while those in another lake might have a low frequency of this allele. Although the genotype frequencies might conform to Hardy–Weinberg predictions within each lake, across the entire range of the fish population, they will not. Geograph-ical subdivision makes the population genetically inhomo-geneous, and such inhomogeneity violates a tacit assump-tion of the Hardy–Weinberg principle: that allele frequencies are uniform throughout the population.

4. *Migration*. When individuals move from one territory to another, they carry their genes with them. The introduction of genes by recent migrants can alter allele and genotype frequencies within a population and disrupt the state of

Hardy–Weinberg equilibrium. As an example, let's consider the situation in **FIGURE 24.2**. Two populations of equal size are separated by a geographical barrier. In population I the frequencies of *A* and *a* are both 0.5, whereas in population II the frequency of *A* is 0.8 and that of *a* is 0.2. With random mating within each population, the Hardy–Weinberg principle predicts that the two populations will have different genotype frequencies (see **FIGURE 24.2**).

Let's suppose that the geographical barrier between the populations breaks down and that the two populations merge completely. In the merged population, the allele frequencies will be the simple averages of the frequencies of the separate populations; the frequency of *A* will be $(0.5 + 0.8)/2 = 0.65$, and the frequency of *a* will be $(0.5 + 0.2)/2 = 0.35$. Moreover, the genotype frequencies in the merged population will be the simple averages of the genotype frequencies in the separate populations: the frequency of *AA* will be $(0.25 + 0.64)/2 = 0.445$, that of *Aa* will be $(0.50 + 0.32)/2 = 0.410$, and that of *aa* will be $(0.25 + 0.04)/2 = 0.145$. Notice, however, that these observed genotype frequencies are not equal to the frequencies predicted by the Hardy–Weinberg principle: $(0.65)^2 = 0.422$ for *AA*, $2(0.65)(0.35) = 0.455$ for *Aa*, and $(0.35)^2 = 0.123$ for *aa*. The reason for this discrepancy is that the observed genotype frequencies were not created by random mating within the entire merged population. Rather, they were created by amalgamating genotype frequencies from separate randomly mating populations. Thus, the merger of two randomly mating populations does not produce a population with Hardy–Weinberg genotype frequencies. However, if the merged population mates randomly for just one generation, Hardy–Weinberg genotype frequencies will be established, and the allele frequencies of the merged population will allow prediction of these genotype frequencies. This example demonstrates that merging randomly mating populations temporarily upsets Hardy–Weinberg equilibrium. The migration of individuals from one population to another also causes a temporary upset in Hardy–Weinberg equilibrium. However, if a population that has received migrants mates randomly for just one generation, Hardy–Weinberg equilibrium will be restored.

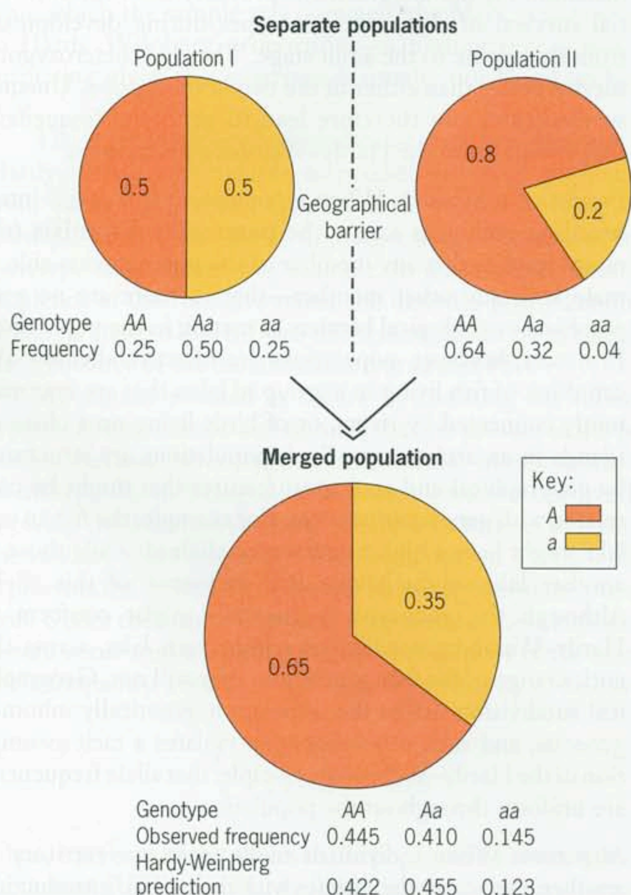


Figure 24.2 ► Effects of population merger on allele and genotype frequencies.

USING ALLELE FREQUENCIES IN GENETIC COUNSELING

Genetic counselors sometimes use allele frequency data in conjunction with pedigree analysis to calculate the risk that an individual will develop a genetic disease. A simple case is shown in **FIGURE 24.3**. The man and woman in generation I have had three children, the last of whom suffered from Tay-Sachs disease, which is caused by an autosomal recessive mutation (*ts*) with a frequency approaching 0.017 in certain populations. Assuming that the frequency of the mutant allele is 0.017 in II-1's ethnic group, her chance of being a carrier (*TS ts*) is obtained by using the Hardy–Weinberg principle: $2\ (0.017)\ (0.983) = 0.033$, which is approximately 1/30. The chance that her husband (II-2) is a carrier is determined by analyzing the pedigree. Because II-4 died of Tay-Sachs disease, we know that both I-1 and I-2 were heterozygous for the mutant allele. Either of them could have transmitted this allele to II-2. However, both of

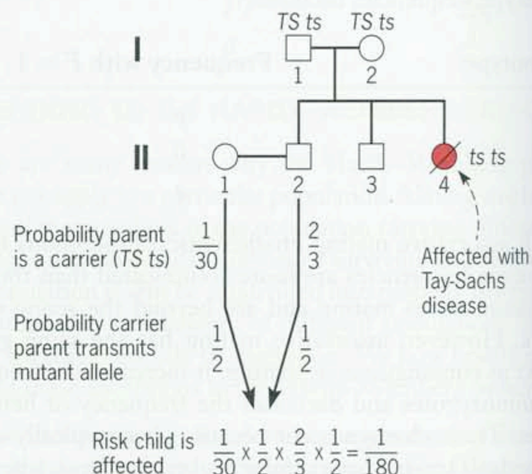


Figure 24.3 ► Pedigree analysis using population data to calculate the risk for Tay-Sachs disease in a child.

:m did not transmit it to him because II-2 does not have the ease. Thus, the chance that II-2 is a carrier of the mutant ele is 2/3. To calculate the risk that II-1 and II-2 will have a ild with Tay-Sachs disease, we combine the probabilities that ch parent is a carrier (1/30 for II-1 and 2/3 for II-2) with the obability that if they are carriers, they will both transmit e mutant allele to their offspring ((1/2) × (1/2) = 1/4). Thus, e risk for the child to have Tay-Sachs disease is (1/30) × (2/3) × 4) = 1/180 = 0.006, which is 20 times the risk for a ndom child in a population where the mutant allele frequency).017.

KEY POINTS

- Allele frequencies can be estimated by enumerating the genotypes in a sample from a population.
- Under the assumption of random mating, the Hardy–Weinberg principle allows genotype frequencies for autosomal and X-linked genes to be predicted from allele frequencies.
- The Hardy–Weinberg principle does not apply to populations with consanguineous or assortative mating, unequal survival among genotypes, geographic subdivision, or migration.
- The Hardy–Weinberg principle is useful in genetic counseling.

Natural Selection

Allele frequencies change systematically in populations because of differential survival and reproduction among genotypes.

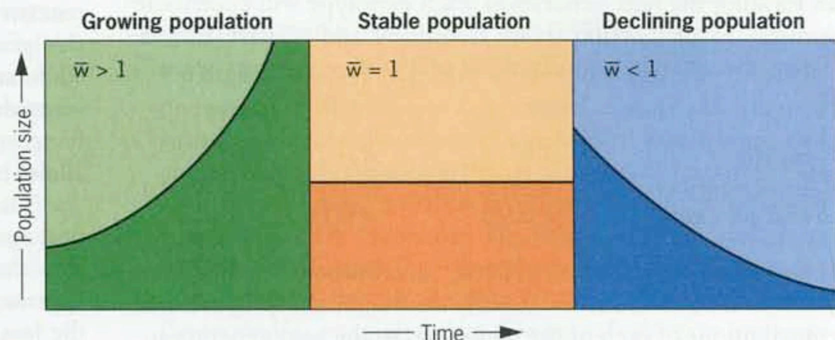
Charles Darwin described the key force that drives evolu- nary change in populations. He argued that organisms pro- ce more offspring than the environment can support and it a struggle for survival ensues. In the face of this competi- n, the organisms that survive and reproduce transmit to their spring traits that favor survival and reproduction. After many nerations of such competition, traits associated with strong mpetitive ability become prevalent in the population, and its associated with weak competitive ability disappear. Selec- n for survival and reproduction in the face of competition is erefore the mechanism that changes the physical and behav- al characteristics of a species. Darwin called this process **tural selection.**

ATURAL SELECTION AT THE LEVEL OF THE GENE

put the mechanism of natural selection into a genetic con- xt, we must recognize that the ability to survive and repro- ce is a phenotype—arguably the most important phenotype all—and that it is determined, at least partly, by genes. eneticists refer to this ability to survive and reproduce as **ness,** a quantitative variable they usually symbolize by the ter w. Each member of a population has its own fitness value: f it dies or fails to reproduce, 1 if it survives and produces 1 offspring, 2 if it survives and produces 2 offspring, and so forth. The average of all these values is the average fitness of the population, usually symbolized $\overline{w}$.

For a population with a stable size, the average fitness is 1; each individual in such a population produces, on average, one offspring. Of course, some individuals will produce more than one offspring, and some will not produce any offspring at all. However, when the population size is not changing, the average number of offspring (that is, the average fitness) is 1. In a declining population, the average number of offspring is less than 1, and in a growing population it is greater than 1 (**FIGURE 24.4**).

To see how fitness differences among individuals lead to change in the characteristics of a population, let's assume that fitness is determined by a single gene segregating two alleles, A and a, in a particular species of insect. Furthermore, let's assume that allele A causes the insects to be dark in color, that allele a causes them to be light in color, and that A is completely dominant to a. In a forest habitat, where plant growth is luxuriant, the dark form of the insect survives better than the light form. Consequently, the fitnesses of genotypes AA and Aa are greater than the fitness of genotype aa. By contrast, in open fields, where plant growth is scarce, the light form of the insect survives better than the dark form, and the fitness relationships are reversed.



gure 24.4 ► Significance of average fitness ($\overline{w}$) for pop- ition size as a function of time. Population size grows, is ble, or declines depending on the value of the average ness.

We can express these relationships mathematically by applying the concept of **relative fitness.** In each of the two environments, we arbitrarily define the fitness of the competitively superior genotype(s) to be equal to 1 and express the fitness of the inferior genotype(s) as a deviation from 1. This fitness deviation, symbolized by the letter s, is called the **selection coefficient;** it measures the intensity of natural selection acting on the genotypes in the population. We can summarize the fitness relationships among the three insect genotypes in each of the two habitats in the following table:

Genotype:	*AA*	*Aa*	*aa*
Phenotype:	dark	dark	light
Relative fitness in forest habitat:	1	1	$1 - s_1$
Relative fitness in field habitat:	$1 - s_2$	$1 - s_2$	1

These relative fitnesses tell us nothing about the absolute reproductive abilities of the different genotypes in the two habitats. However, they do tell us how well each genotype competes with the other genotypes within a particular environment. Thus, for example, we know that *aa* is a weaker competitor than either *AA* or *Aa* in the forest habitat. How much weaker depends, of course, on the actual value of the selection coefficient, s_1. If $s_1 = 1$, then *aa* is effectively a lethal genotype (its relative fitness is 0), and we would expect natural selection to reduce the frequency of the *a* allele from the population. If s_1 were much smaller, say only 0.01, natural selection would still reduce the frequency of the *a* allele, but it would do so very slowly.

To see the effect of natural selection on allele frequencies, let's focus on an insect population in the forest habitat. We shall assume that initially the frequency of *A* is $p = 0.5$, that the frequency of *a* is $q = 0.5$, and that $s_1 = 0.1$. Furthermore, let's assume that the population mates randomly and that the genotypes are present in Hardy–Weinberg frequencies at fertilization each generation. (Differential survival among the genotypes will change these frequencies as the insects mature.) Under these assumptions, the initial genetic composition of the population is:

Genotype:	*AA*	*Aa*	*aa*
Relative fitness:	1	1	$1 - 0.1 = 0.9$
Frequency: (at fertilization)	$q^2 = 0.25$	$p^2 = 0.25$	$2pq = 0.50$

In forming the next generation, each genotype will contribute gametes in proportion to its frequency and relative fitness. Thus, the relative contributions of the three genotypes will be:

Genotype:	*AA*	*Aa*	*aa*
Relative contribution to next generation:	$(0.25) \times (0.9) = 0.225$	$(0.25) \times 1 = 0.25$	$(0.50) \times 1 = 0.50$

If we divide each of these relative contributions by their sum ($0.25 + 0.50 + 0.225 = 0.975$), we obtain the proportional contributions of each of the genotypes to the next generation:

Genotype:	*AA*	*Aa*	*aa*
Proportional contribution to next generation:	0.513	0.231	0.2

From these numbers we can calculate the frequency of th *a* allele after one generation of selection simply by noting th all the genes transmitted by the *aa* homozygotes are *a* and th half the genes transmitted by the *Aa* heterozygotes are *a*. In th next generation, the frequency of *a*, symbolized q', will be

$$q' = 0.231 + (1/2)(0.513) = 0.487$$

which is slightly less than the starting frequency of 0.5. Thu in the forest habitat, natural selection, acting through the low fitness of the *aa* homozygotes, has decreased the frequency of from 0.5 to 0.487. In every subsequent generation, the fr quency of *a* will be reduced slightly because of selection again the *aa* homozygotes, and eventually, this allele will be elim nated from the population altogether. **FIGURE 24.5*a*** shows ho natural selection will drive the *a* allele to extinction.

In the field habitat, *aa* homozygotes are selectively sup rior to the other two genotypes. Thus, starting with $q = 0.$ Hardy–Weinberg genotype frequencies, and the selection coe ficient $s_2 = 0.1$, we have:

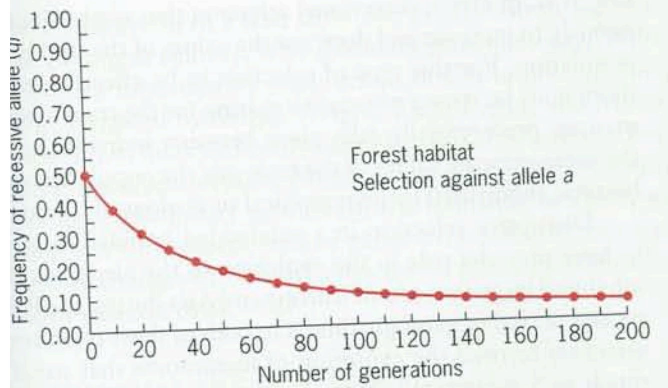
Genotype:	*AA*	*Aa*	*aa*
Relative fitness:	$1 - 0.1 = 0.9$	$1 - 0.1 = 0.9$	1
Frequency:	0.25	0.50	0.2

After one generation of selection in the field habitat, the fr quency of *a* will be $q' = 0.513$, which is slightly greater than th starting frequency. Every generation afterward, the frequenc of *a* will rise, and eventually it will equal 1, at which point it said that the allele has been fixed in the population. **FIGUR 24.5*b*** shows the selection-driven path toward fixation of *a*.

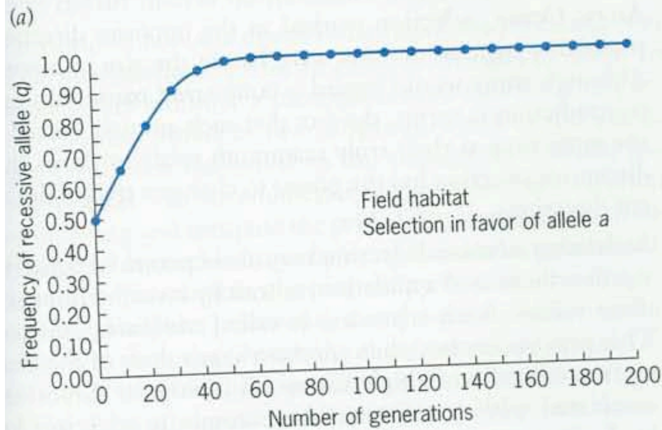
These two scenarios illustrate selection for or against recessive allele. In the forest habitat, the recessive allele *a* deleterious in homozygous condition and selection acts again it. In the field habitat, *a* is selectively favored over the dominar allele *A*, which is deleterious in both homozygous and hetero zygous condition.

Notice that selection *for* a recessive allele—and therefor against a harmful dominant allele—is more effective than selec tion *against* a recessive allele. The curve in **FIGURE 24.5*b*** show the time course of selection in favor of a recessive allele. Thi curve rises steeply to the top of the graph, at which point th recessive allele is fixed in the population. The process shown i this graph efficiently changes the frequency of the recessiv allele and rather quickly gets it to a final value of 1, becaus every dominant allele in the population is exposed to the puri fying action of selection. By virtue of their dominance, thes alleles cannot "hide out" in heterozygous condition.

The curve in **FIGURE 24.5a** shows the time course of selec tion against a recessive allele. This curve changes more gradu ally than the curve in **FIGURE 24.5*b*** and asymptoticall approaches a limit at the bottom of the graph, which represent the loss of the recessive allele. Selection is less effective in thi



(a)



(b)

Figure 24.5 ▶ (*a*) Selection against the recessive allele *a* in the forest habitat. (*b*) Selection in favor of the recessive allele *a* in the field habitat.

case because it can only act against the recessive allele when it is homozygous. Once the recessive allele has been reduced in frequency, recessive homozygotes will be rare; most of the surviving recessive alleles will therefore be found in heterozygotes, where they are immune from the purifying effect of selection. By comparing the two graphs in **FIGURE 24.5**, we see that a harmful recessive allele can linger in a population much longer than a harmful dominant allele.

Studies of the moth *Biston betularia*, an inhabitant of wooded areas in Great Britain, have shown that selection of the type we have been discussing does operate to change allele frequencies in nature. This species, commonly known as the peppered moth, exists in two color forms, light and dark (**FIGURE 24.6**); the light form is homozygous for a recessive allele *c*, and the dark form carries a dominant allele *C*. From 1850 onward, the frequency of the dark form increased in certain areas of England, particularly in the industrialized Midlands section of the country. Around the heavily industrialized cities of Manchester and Birmingham, for example, the frequency of the dark form increased from 1 to 90 percent. This dramatic increase has been attributed to selection against the light form in the soot-polluted landscapes of industrialized areas. In recent times, the level of pollution has abated considerably and the light form of the moth has made a comeback, although not quite to its preindustrial frequencies. Whatever processes have been at work against the light form of the moth appear to have been reversed by environmental restoration in this region of England.

(a)

(b)

Figure 24.6 ▶ (*a*) The dark form of the peppered moth on tree bark covered with lichens. (*b*) The light form of the peppered moth on tree bark covered with soot from industrial pollution.

NATURAL SELECTION AT THE LEVEL OF THE PHENOTYPE

Although the example of *Biston betularia* shows that fitness can be dramatically influenced by different alleles of a single gene, in most circumstances it is influenced by the alleles of many genes. Typically, fitness depends on sets of genes that control quantitative traits such as body size, disease susceptibility, and fecundity. Thus, we would expect natural selection to affect the statistical distributions of these kinds of traits within a population. We now consider three ways in which selection can affect the distribution of a quantitative trait (**FIGURE 24.7**).

1. *Directional selection*. Selection that favors values of a trait at one end of its distribution is *directional selection*. This type of situation commonly occurs in agriculture where plant and animal breeders practice artificial selection to improve traits such as crop yield, nutritional content, and egg production (see Chapter 23). In nature, directional selection may occur when a deteriorating environment steadily challenges the population to adapt. R. A. Fisher, who studied this situation theoretically, concluded that directional selection increases

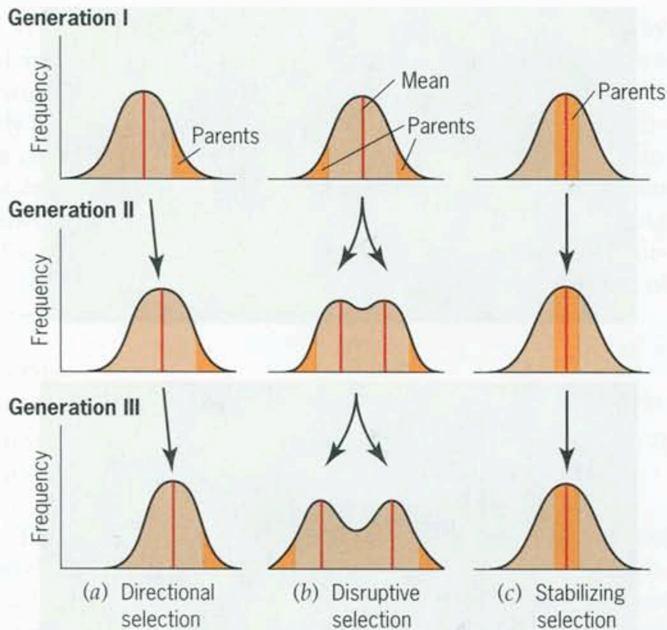


Figure 24.7 ▶ The effects of directional, disruptive, and stabilizing selection on the frequency distribution of a quantitative trait. The mean of the trait is indicated by a red line.

the average fitness of a population at a rate that is proportional to the additive genetic variance for fitness—a principle that he immodestly called the "fundamental theorem of natural selection." Although a discussion of Fisher's theorem is beyond the scope of this textbook, it should be noted that the theorem parallels the principle that the response to artificial selection depends on the proportion of variance in a trait that is additive genetic variance—that is, it depends on the narrow-sense heritability (see Chapter 23). Thus, the rate at which natural selection can change fitness in a population is a function of the narrow-sense heritability for fitness.

Evolutionary biologists have discovered many examples of directional selection. The increase in body size of the horse during the last 40 million years, the development of extravagant body ornaments such as antlers in deer and feathers in birds, and the increase in brain size in our own species all probably involved directional selection.

2. *Disruptive selection*. Selection that favors extreme values of a trait at the expense of intermediate values is *disruptive selection*. It is, in effect, directional selection that works simultaneously to increase and decrease the values of the trait in the population. For this type of selection to be effective, either there must be strong assortative mating for the trait—that is, matings preferentially take place between individuals with the same extreme values of the trait—or the population must become subdivided by geographical or ecological barriers.

   Disruptive selection in a subdivided population seems to have played a role in the evolution of the elephants that inhabited large sections of Europe and Asia during the Pleistocene period of geologic time. On both of these continents, selection favored the evolution of mammoths that stood as much as 5 meters tall. However, on certain islands in the Arctic Ocean, selection worked in the opposite direction, producing mammoths that were barely the size of a pony. Although some would regard a pony-sized mammoth as a contradiction in terms, the fact that such animals existed at the same time as their truly mammoth relatives shows that disruptive selection has the power to change a trait in different directions.

3. *Stabilizing selection*. Selection may also operate to conserve the distribution of a quantitative trait by favoring intermediate values. Such a process is called *stabilizing selection*. This process occurs when intermediate values of the trait are associated with high fitness and extreme values are associated with low fitness. An example is selection for birth weight in human babies. The optimum birth weight is around 8 pounds. Babies that deviate significantly from this weight are less likely to survive; larger ones may be injured during birth, and smaller ones are more likely to die after birth.

KEY POINTS

- Natural selection occurs when genotypes differ in the ability to survive and reproduce—that is, when they differ in fitness.
- The intensity of natural selection is quantified by the selection coefficient.
- At the level of the gene, natural selection changes the frequencies of alleles in populations.
- At the level of the phenotype, natural selection influences the distributions of quantitative traits.
- Natural selection may be directional, disruptive, or stabilizing.

Random Genetic Drift

Allele frequencies change unpredictably in populations because of uncertainties during reproduction.

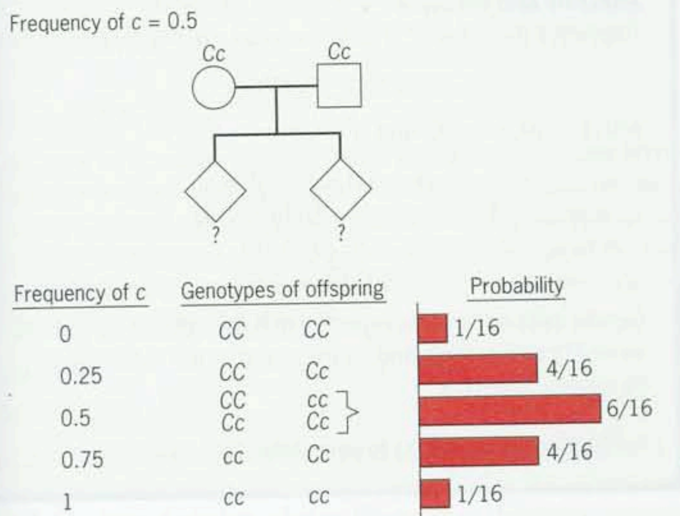
In his book *The Origin of Species*, Darwin emphasized the role of natural selection as a systematic force in evolution. However, he also recognized that evolution is affected by random processes. New mutants appear unpredictably in populations. Thus, mutation, the ultimate source of all genetic variability, is a random process that profoundly affects evolution; without mutation, evolution could not occur. Darwin also recognized that inheritance (which he did not understand) is unpredictable. Traits are inherited, but offspring are not exact replicas of their parents; there is always some unpredictability

he transmission of a trait from one generation to the next. he twentieth century, after Mendel's principles were redis- ered, the evolutionary implications of this unpredictability e investigated by Sewall Wright, R. A. Fisher, and Motoo nura. From their theoretical analyses, it is clear that the ran- nness associated with the Mendelian mechanism profoundly cts the evolutionary process. In the following sections, we lore how the uncertainties of genetic transmission can lead andom changes in allele frequencies—a phenomenon called **dom genetic drift.**

NDOM CHANGES IN ALLELE FREQUENCIES

investigate how the uncertainties associated with the Men- ian mechanism can lead to random changes in allele fre- encies, let's consider a mating between two heterozygotes, × *Cc*, that produces two offspring, which is the number ected if each individual in the population replaces itself **GURE 24.8**). We can enumerate the possible genotypes of the o offspring and compute the probability associated with each the possible combinations by using the methods discussed in apter 3. For example, the probability that the first offspring *CC* is 1/4 and the probability that the second offspring is *CC* also 1/4; thus, the probability that both offspring are *CC* is 4) × (1/4) = 1/16. The probability that one of the offspring *CC* and the other is *Cc* is (1/4) × (1/2) × 2 (because there are o possible birth orders: *CC* then *Cc*, or *Cc* then *CC*); thus, the obability of observing the genotypic combination *CC* and *Cc* the two offspring is 1/4. The entire probability distribution r the various genotypic combinations of offspring is given in **GURE 24.8**. This figure also gives the frequency of the *c* allele sociated with each combination.

Among the parents, the frequency of *c* is 0.5. This fre- iency is the most probable frequency for *c* among the two fspring. In fact, the probability that the frequency of *c* will not change between parents and offspring is 6/16. However, there is an appreciable chance that the frequency of *c* will increase or decrease among the offspring simply because of the uncertain- ties associated with the Mendelian mechanism. The chance that the frequency of *c* will increase is 5/16, and the chance that it will decrease is also 5/16. Thus, the chance that the frequency of *c* will change in one direction or the other, 5/16 + 5/16 = 10/16, is actually greater than the chance that it will remain the same.



igure 24.8 ► Probabilities associated with possible frequencies of he allele *c* among the two children of heterozygous parents.

This situation illustrates the phenomenon of random genetic drift. For every pair of parents in the population that is segregating different alleles of a gene, there is a chance that the Mendelian mechanism will lead to changes in the frequencies of those alleles. When these random changes are summed over all pairs of parents, there may be aggregate changes in the allele frequencies. Thus, the genetic composition of the population can change even without the force of natural selection.

Random genetic drift is essentially the result of a gene sampling process that occurs when organisms reproduce. This sampling process has two components. First, the alleles of seg- regating genes are randomly incorporated into gametes. The offspring produced by a heterozygote with genotype *Cc* inherit either allele *C* or allele *c*, each with probability 1/2. Thus, in segregating individuals, there is always uncertainty as to which allele a given offspring will receive. Second, there is random variation in the number of offspring that a parent produces. Some parents produce many offspring, some produce a few, and some produce none. Although part of this variation may be due to intrinsic fitness differences among the members of the population, part of it may be due to purely random factors— accidental deaths, bad weather, environmental catastrophes. This purely random variation in reproductive output com- pounds the randomness associated with Mendelian segrega- tion, and the net result is random change in allele frequencies.

THE EFFECTS OF POPULATION SIZE

A population's susceptibility to random genetic drift depends on its size. In large populations, the effect of genetic drift is minimal, whereas in small ones, it may be the primary evolu- tionary force. Geneticists gauge the effect of population size by monitoring the frequency of heterozygotes over time. Let's focus, once again, on alleles *C* and *c*, with respective frequen- cies p and q, and let's assume that neither allele has any effects on fitness; that is, *C* and *c* are selectively neutral. Furthermore, let's assume that the population mates randomly and that in any given generation, the genotypes are present in Hardy– Weinberg proportions.

In a very large population—essentially infinite in size—the frequencies of *C* and *c* will be constant, and the frequency of the heterozygotes that carry these two alleles will be $2pq$. In a small population of finite size N, the allele frequencies will change randomly as a result of genetic drift. Because of these changes, the frequency of heterozygotes, often called the **heterozygosity**, will also change. To express the magnitude of this change over

one generation, let's define the current frequency of heterozygotes as H and the frequency of heterozygotes in the next generation as H'. Then the mathematical relationship between H' and H is

$$H' = \left(1 - \frac{1}{2N}\right) H$$

This equation tells us that in one generation, random genetic drift causes the heterozygosity to decline by a factor of $1/2N$. In a total of t generations, we would expect the heterozygosity to decline to a level given by the equation

$$H_t = \left(1 - \frac{1}{2N}\right)^t H$$

This equation enables us to see the cumulative effect of random genetic drift over many generations. In each generation, the heterozygosity is expected to decline by a factor of $1/2N$; over many generations, the heterozygosity will eventually be reduced to 0, at which point all genetic variability in the population will be lost. At this point the population will possess only one allele of the gene, and either $p = 1$ and $q = 0$, or $p = 0$ and $q = 1$. Thus, through random changes in allele frequencies, drift steadily erodes the genetic variability of a population, ultimately leading to the fixation and loss of alleles. It is important to recognize that this process depends critically on the population size (**FIGURE 24.9**). Small populations are the most sensitive to the variability-reducing effects of drift. Large populations are less sensitive. To see how drift might have reduced genetic variability in the population of Pitcairn Island described at the beginning of this chapter, work through the Focus on Problem Solving: Applying Genetic Drift to Pitcairn Island.

If selectively neutral alleles of the sort we have been discussing are ultimately destined for fixation or loss, can we determine the probabilities that are associated with these two ultimate outcomes? Let's suppose that at the current time, the frequency of C is p and that of c is q. Then, as long as the alleles

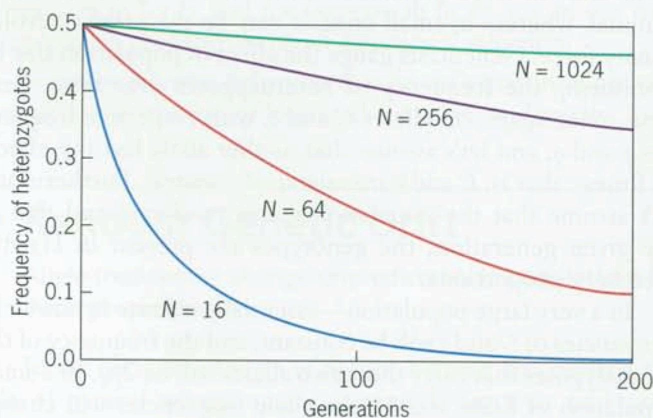


Figure 24.9 ▶ Decline in the frequency of heterozygotes due to random genetic drift in populations of different size N. The populations begin with $p = q = 0.5$.

▶ FOCUS ON PROBLEM SOLVING
Applying Genetic Dri
to Pitcairn Island

THE PROBLEM

When Fletcher Christian and his fellow mutineers on H.M.S. *Bounty* settled on Pitcairn Island, they didn't rea that they were beginning a genetic experiment. The found group of men and women brought a finite sample of gene the island—a sample from two larger populations, Britain a Polynesia. From its beginning in 1790, the Pitcairn Island co ny has essentially been a closed system. Some people have the island, but very few have migrated to it. Most of the alle that are present on the island today are copies of alleles t were brought there by the colony's founders. Of course, every allele that was present at the founding is present tod Some alleles were lost through the death or infertility of th carriers. Others have been lost through genetic drift. Let's s pose that the average population size of Pitcairn Island been 20 and that when the colony was founded, H (the h erozygosity) was 0.20. Let's also suppose that 10 generati have elapsed since the founding of the colony. What is expected value of H today?

FACTS AND CONCEPTS

1. The heterozygosity is a measure of genetic variability i population.

2. In a population of size N, genetic drift is expected to red the heterozygosity by a factor of $1/2N$ each generation.

3. The loss in variability is cumulative; after t generations, heterozygosity is given by $H_t = (1 - 1/2N)^t H$.

ANALYSIS AND SOLUTION

To predict the value of H today, we can use the equation

$$H_t = (1 - 1/2N)^t H$$

with $t = 10$, $N = 20$, and $H = 0.20$

$$\begin{aligned} H_{10} &= (1 - 1/2N)^{10} H \\ &= (1 - 1/40)^{10}\,(0.20) \\ &= (0.78)(0.20) \\ &= 0.15 \end{aligned}$$

Genetic drift is therefore expected to have reduced the gene variability on Pitcairn Island, as measured by the heterozygos by about 25 percent.

For further discussion go to your *WileyPLUS* course.

are selectively neutral and the population mates randomly, the probability that a particular allele will ultimately be fixed in the population is its current frequency—p for allele C and q for allele c—and the probability that the allele will ultimately be lost from the population is 1 minus its current frequency; that is, $1 - p$ for allele C and $1 - q$ for allele c. Thus, when random genetic drift is the driving force in evolution, we can assign specific probabilities to the possible evolutionary outcomes and, remarkably, these probabilities are independent of population size.

KEY POINTS

- Genetic drift, the random change of allele frequencies in populations, is due to uncertainties in Mendelian segregation and to unpredictable variation in the number of offspring.
- In diploid organisms, the rate at which genetic variability is lost by random genetic drift is $1/2N$, where N is the population size.
- Small populations are more susceptible to drift than large ones.
- Drift ultimately leads to the fixation of one allele at a locus and the loss of all other alleles; the probability that an allele will ultimately be fixed is equal to its current frequency in the population.

Populations in Genetic Equilibrium

The evolutionary forces of mutation, selection, and drift may oppose each other to create a dynamic equilibrium in which allele frequencies no longer change.

In a randomly mating population without selection or drift to change allele frequencies, and without migration or mutation to introduce new alleles, the Hardy–Weinberg genotype frequencies persist indefinitely. Such an idealized population is in a state of genetic equilibrium. In reality, the situation is much more complicated; selection and drift, migration and mutation are almost always at work changing the population's genetic composition. However, these evolutionary forces may act in contrary ways to create a *dynamic equilibrium* in which there is no net change in allele frequencies. This type of equilibrium differs fundamentally from the equilibrium of the ideal Hardy–Weinberg population. In a dynamic equilibrium, the population simultaneously tends to change in opposite directions, but these opposing tendencies cancel each other and bring the population to a point of balance. In the ideal Hardy–Weinberg equilibrium, the population does not change because there are no evolutionary forces at work. We now explore how opposing evolutionary forces can create a dynamic equilibrium within a population.

BALANCING SELECTION

One type of dynamic equilibrium arises when selection favors the heterozygotes at the expense of each type of homozygote in the population. In this situation, called *balancing selection* or *heterozygote advantage*, we can assign the relative fitness of the heterozygotes to be 1 and the relative fitnesses of the two types of homozygotes to be less than 1:

Genotype:	AA	Aa	aa
Relative fitness:	$1 - s$	1	$1 - t$

In this formulation, the terms $1 - s$ and $1 - t$ contain selection coefficients that are assumed to lie between 0 and 1. Thus, each of the homozygotes has a lower fitness than the heterozygotes. The superiority of the heterozygotes is sometimes referred to as *overdominance*.

In cases of heterozygote advantage, selection tends to eliminate both the A and a alleles through its effects on the homozygotes, but it also preserves these alleles through its effects on the heterozygotes. At some point these opposing tendencies balance each other, and a dynamic equilibrium is established. To determine the frequencies of the two alleles at the point of equilibrium, we must derive an equation that describes the process of selection, and then solve this equation for the allele frequencies when the opposing selective forces are in balance—that is, when the allele frequencies are no longer changing (**TABLE 24.2**). At the balance point, the frequency of A is $p = t/(s + t)$, and the frequency of a is $q = s/(s + t)$.

As an example, let's suppose that the AA homozygotes are lethal ($s = 1$) and that the aa homozygotes are 50 percent as fit as the heterozygotes ($t = 0.5$). Under these assumptions, the population will establish a dynamic equilibrium when $p = 0.5/(0.5 + 1) = 1/3$ and $q = 1/(0.5 + 1) = 2/3$. Both alleles will be maintained at appreciable frequencies by selection in favor of the heterozygotes—a condition known as a **balanced polymorphism.**

TABLE 24.2

Calculating Equilibrium Allele Frequencies with Balancing Selection

Genotypes:	AA	Aa	aa
Relative fitnesses:	$1 - s$	1	$1 - t$
Frequencies:	p^2	$2pq$	q^2

Average relative fitness: $\bar{w} = p^2 \times (1 - s) + 2pq \times 1 + q^2 (1 - t)$

Frequency of A in the next generation after selection:

$$p' = [p^2(1 - s) + (1/2)2pq]/\bar{w} = p(1 - sp)/\bar{w}$$

Change in frequency of A due to selection:

$$\Delta p = p' - p = pq(tq - sp)/\bar{w}$$

At equilibrium, $\Delta p = 0$: $p = t/(s + t)$ and $q = s/(s + t)$

In humans, the disease sickle-cell anemia is associated with a balanced polymorphism. Individuals with this disease are homozygous for a mutant allele of the β-globin gene, denoted HBB^S, and they suffer from a severe form of anemia in which the hemoglobin molecules crystallize in the blood. This crystallization causes the red blood cells to assume a characteristic sickle shape. Because sickle-cell anemia is usually fatal without medical treatment, the fitness of HBB^SHBB^S homozygotes has historically been 0. However, in some parts of the world, particularly in tropical Africa, the frequency of the HBB^S allele is as high as 0.2. With such harmful effects, why does the HBB^S allele remain in the population at all?

The answer is that there is moderate selection against homozygotes that carry the wild-type allele HBB^A. These homozygotes are less fit than the HBB^SHBB^A heterozygotes because they are more susceptible to infection by the parasites that cause malaria, a fitness-reducing disease that is widespread in regions where the frequency of the HBB^S allele is high (**FIGURE 24.10**). We can schematize this situation by assigning relative fitnesses to each of the genotypes of the β-globin gene:

Genotype:	HBB^SHBB^S	HBB^SHBB^A	HBB^AHBB^A
Relative fitness:	$1-s$	1	$1-t$

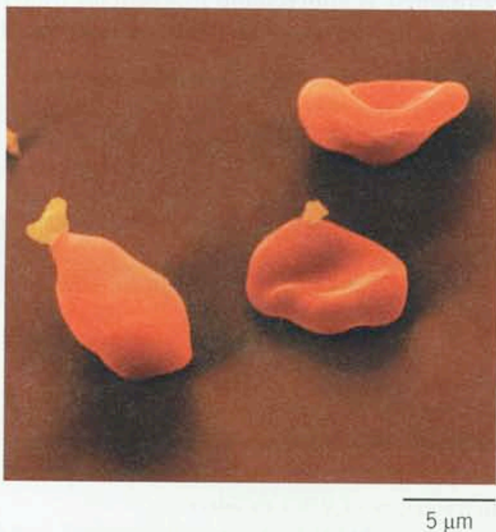
If we assume that the equilibrium frequency of HBB^S is $p =$ 0.1—a typical value in West Africa—and if we note that $s = 1$ because the HBB^SHBB^S homozygotes die, we can estimate the intensity of selection against the HBB^AHBB^A homozygotes because of their greater susceptibility to malaria:

$$p = t / (s + t)$$

$$0.1 = t / (1 + t)$$

$$t = (0.1) / (0.9) = 0.11$$

This result tells us that the HBB^AHBB^A homozygotes are about 11 percent less fit than the HBB^SHBB^A heterozygotes. Thus, the selective inferiority of the HBB^SHBB^S and HBB^AHBB^A homozygotes compared to the heterozygotes creates a balanced polymorphism in which both alleles of the β-globin gene are maintained in the population.



5 µm

Figure 24.10 ▸ The malaria parasite *Plasmodium falciparum* (yellow) emerging from red blood cells that it had infected.

Various other mutant *HBB* alleles are found at appreciable frequencies in tropical and subtropical regions of the world in which *falciparum* malaria is—or was—endemic. It is plausible that these alleles have also been maintained in human populations by balancing selection.

MUTATION–SELECTION BALANCE

Another type of dynamic equilibrium is created when selection eliminates deleterious alleles that are produced by recurrent mutation. For example, let's consider the case of a deleterious recessive allele *a* that is produced by mutation of the wild-type allele *A* at rate u. A typical value for u is 3×10^{-6} mutations per generation. Even though this rate is very low, over time, the mutant allele will accumulate in the population, and, because it is recessive, it can be carried in heterozygous condition without having any harmful effects. At some point, however, the mutant allele will become frequent enough for *aa* homozygotes to appear in the population, and these will be subject to the force of selection in proportion to their frequency and the value of the selection coefficient s. Selection against these homozygotes will counteract the force of mutation, which introduces the mutant allele into the population.

If we assume that the population mates randomly, and if we denote the frequency of *A* as p and that of *a* as q, then we can summarize the situation as follows:

Mutation:		Selection:		
produces *a*		eliminates *a*		
$A \rightarrow a$	Genotype:	*AA*	*Aa*	*aa*
rate = u	Relative fitness:	1	1	$1-s$
	Frequency:	p^2	$2pq$	q^2

Mutation introduces mutant alleles into the population at rate u, and selection eliminates them at rate sq^2 (**FIGURE 24.11**). When these two processes are in balance, a dynamic equilibrium will be established. We can calculate the frequency of the mutant allele at the equilibrium created by mutation–selection balance by equating the rate of mutation to the rate of elimination by selection:

$$u = sq^2$$

Thus, after solving for q, we obtain

$$q = \sqrt{u/s}$$

For a mutant allele that is lethal in homozygous condition, $s = 1$, and the equilibrium frequency of the mutant allele is simply the square root of the mutation rate. If we use the value for u that was given above, then for a recessive lethal allele the equilibrium frequency is $q = 0.0017$. If the mutant allele is not

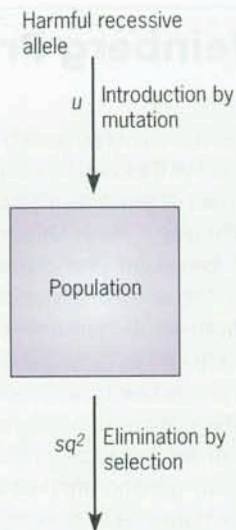


Figure 24.11 Mutation–selection balance for a deleterious recessive allele with frequency q. Genetic equilibrium is reached when the introduction of the allele into the population by mutation at rate u is balanced by the elimination of the allele by selection with intensity s against the recessive homozygotes.

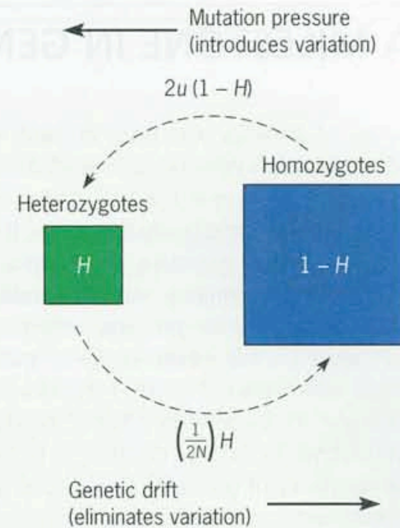


Figure 24.12 Mutation–drift balance for variability as measured by the frequency of heterozygotes H in a population of size N. An equilibrium frequency of heterozygotes is reached when the introduction of variability by mutation at rate u is balanced by the elimination of variability by genetic drift at rate $\frac{1}{2N}$.

completely lethal in homozygous condition, then the equilibrium frequency will be higher than 0.0017 by a factor that depends on $1/\sqrt{s}$. For example, if s is 0.1, then at equilibrium the frequency of this slightly deleterious allele will be $q = 0.0055$, or 3.2 times greater than the equilibrium frequency of a recessive lethal allele.

Studies with natural populations of *Drosophila* have indicated that lethal alleles are less frequent than the preceding calculations predict. The discrepancy between the observed and predicted frequencies has been attributed to partial dominance of the mutant alleles—that is, these alleles are not completely recessive. Natural selection appears to act against deleterious alleles in heterozygous condition as well as in homozygous condition. Thus, the equilibrium frequencies of these alleles are lower than we would otherwise predict. Selection that acts against mutant alleles in homozygous or heterozygous condition is sometimes called *purifying selection*.

MUTATION–DRIFT BALANCE

We have already seen that random genetic drift eliminates variability from a population. Without any counteracting force, this process would eventually make all populations completely homozygous. However, mutation replenishes the variability that is lost by drift. At some point, the opposing forces of mutation and genetic drift come into balance and a dynamic equilibrium is established.

Previously, we saw that genetic variability can be quantified by calculating the frequency of heterozygotes in a population—a statistic called the heterozygosity, which is symbolized by the letter H. The frequency of homozygotes in a population—often called the *homozygosity*—is equal to $1 - H$. Over time, genetic drift decreases H and increases $1 - H$, and mutation does just the opposite (**FIGURE 24.12**). Let's assume that each new mutation is selectively neutral. In a randomly mating population of size N, the rate at which drift decreases H is $(\frac{1}{2N})\, H$ (see the earlier section, The Effects of Population Size). The rate at which mutation increases H is proportional to the frequency of the homozygotes in the population $(1 - H)$ and the probability that one of the two alleles in a particular homozygote mutates to a different allele, thereby converting that homozygote into a heterozygote. This probability is simply the mutation rate u for each of the two alleles in the homozygote; thus, the total probability of mutation converting a particular homozygote into a heterozygote is $2u$. The rate at which mutation increases H in a population is therefore equal to $2u(1 - H)$.

When the opposing forces of mutation and drift come into balance, the population will achieve an equilibrium level of variability denoted by $\hat{H}$. We can calculate this equilibrium value of H by equating the rate at which mutation increases H to the rate at which drift decreases it:

$$2u(1 - H) = \left(\frac{1}{2N}\right)H$$

By solving for H, we obtain the equilibrium heterozygosity at the point of mutation–drift balance:

$$\hat{H} = 4Nu\,/\,(4Nu + 1)$$

Thus, the equilibrium level of variability (as measured by the heterozygosity) is a function of the population size and the mutation rate.

If we assume that the mutation rate is $u = 1 \times 10^{-6}$, we can plot $\hat{H}$ for different values of N (**FIGURE 24.13**). For $N < 10{,}000$,

▶ A MILESTONE IN GENETICS: The Hardy–Weinberg Principle

The modern science of genetics was born in 1866 when Gregor Mendel published his paper on inheritance in peas. Because Mendel's paper appeared in an obscure journal, it initially had no impact. Thirty-four years elapsed before the world finally recognized the significance of Mendel's discoveries. After Mendel's ideas came to light, the science of genetics developed quickly. Various subdisciplines were born—for example, biochemical genetics, which started with Archibald Garrod's work on the inborn errors of metabolism, and *Drosophila* genetics, which started when T. H. Morgan found the white-eye mutant in one of his laboratory cultures. Population genetics also began about this time. In fact, we can date its birth to 1908, the year in which the constancy of genotype frequencies under random mating was first described.

The distribution of genotype frequencies was explored in two articles, one published in the high-profile American journal *Science* and the other in the annual volume of the Society for Natural History in Württemberg, Germany—a publication that was not too widely read. G. H. Hardy, an eminent British mathematician, was the author of the *Science* paper. Wilhelm Weinberg, a German physician, was the author of the paper published in the Württemberg annual. Hardy and Weinberg arrived at their conclusions independently, and today, we refer to their discovery about genotype frequencies as the Hardy–Weinberg principle: If A and a are alleles with frequencies p and q, respectively, then in a large population with random mating and without selection, the frequencies of the three genotypes are p^2 (AA), $2pq$ (Aa), and q^2 (aa). Furthermore, these frequencies will persist generation after generation—that is, the population will remain in a state of genetic equilibrium.

Hardy was prompted to write his short paper[1] describing this principle in response to remarks made by Udny Yule, who had suggested that under Mendelism a dominant trait should eventually be expressed in three-fourths of the members of a population. Yule had a particular dominant trait, brachydactyly or short fingers, in mind, and he obviously knew that brachydactyly is not manifested in three-fourths of the human population. Using "a little mathematics of the multiplication-table type," Hardy showed that the frequencies of genotypes and their associated phenotypes are stable from one generation to the next as long as mating is random and the population is reasonably large. Thus, he demonstrated that a trait such as brachydactyly should not increase in frequency simply because it is dominant, as Yule had conjectured. Hardy did note that from one generation to the next there might be small fluctuations in genotype frequencies on account of the finite size of the population. Thus, he anticipated the concept of genetic drift, which was analyzed two decades later by Sewall Wright and R. A. Fisher.

Hardy regarded his paper as utterly trivial and may have published it in an American journal to minimize the chance that his British colleagues would see it. He was "a pure mathematician's pure mathematician. He abhorred any 'practical' mathematics. For him, pure mathematics was beautiful and useless, while useful mathematics was dull and ugly. It must have embarrassed him that his mathematically most trivial paper is not only far and away his most widely known, but has been of such distastefully practical value."[2]

Weinberg wrote his paper[3] to investigate whether or not the tendency for women to produce twins is determined by a Mendelian factor. His interest in twinning is not surprising because as a physician he attended at more than 3500 births. Despite a busy medical practice, he had time to read and think about heredity, and he looked for ways to apply Mendelian concepts to human traits. The title of his article translates as "On the Demonstration of Inheritance in Humans."

Weinberg recognized that pedigree analysis provides one way to study human heredity; however, the trait that interested him—

[1]Hardy, G. H. 1908. Mendelian proportions in a mixed population. *Science* 28: 49–50.

[2]Crow, J. F. 1988. Eighty years ago. The beginnings of population genetics. *Genetics* 119:473–476.

[3]Weinberg, W. 1908. Über den Nachweis der Vererbung beim Menschen. *Jahreshefte Vereins für vaterländische Naturkunde in Württemberg* 64:369–382.

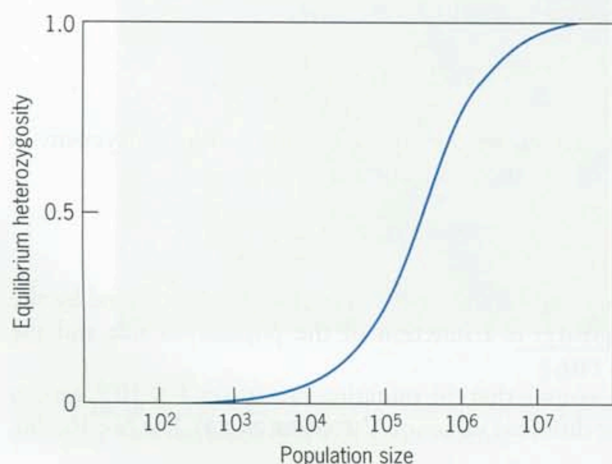


Figure 24.13 ▶ Equilibrium frequency of heterozygotes (heterozygosity) under mutation–drift balance as a function of genetically effective population size. The mutation rate is assumed to be 10^{-6}.

/inning—was not amenable to conventional pedigree analysis. As ı alternative, he took a broader approach, which involved ascertaining e frequencies of traits (and their underlying Mendelian determinants) whole populations. One fact suggested to him that twinning is ɛritable. The frequency of dizygotic twins varies among different hnic groups—for example, it is higher among Germans than among ılians. The mathematical relationship between allele frequencies ıd genotype frequencies that we now know as the Hardy-Weinberg 'inciple provided Weinberg with a theoretical foundation on which build a methodology for his genetic studies. However, his analysis as not definitive; the best he could do was to suggest that twinning humans is due to a recessive allele—clearly an oversimplification for ıch a complex trait.

Until the 1940s, the Hardy–Weinberg principle was known as ardy's law in the English-speaking world. Curt Stern, a geneticist 'ho fled Nazi Germany to work in America, added Weinberg's name the law by publishing a note in *Science* in 1943.[4] Stern translated ıe relevant passages of Weinberg's 1908 paper and offered an ‹planation for why the paper had been largely ignored:

> While Weinberg's paper, like Mendel's, appeared in an obscure journal, its failure to be recognized can not be ascribed to this fact alone. His later contributions dealing with extensions of the statistical treatment of the genetics of populations are found in the "regular" journals. These papers have received some attention and in them Weinberg refers to his 1908 pioneer work. However, both Weinberg and Hardy were ahead of contemporary thought and similar problems were not generally considered for at least eight years. At that time perhaps Hardy's name and the prominent place of his publication both helped to leave Weinberg's contribution neglected.[5]

Stern also made a proposal, which the scientific community has accepted:

> Hardy as a mathematician did not follow up his discovery by any further consideration of its genetic implications. Weinberg in 1909 reformulated his theorem in terms valid for multiple alleles—at a time when no case of multiple alleles had been discovered in man. He also for the first time investigated polyhybrid populations and recognized their essentially different method of attaining equilibrium. Considering these facts it seems a matter of justice to attach the names of both the discoverers to the population formula.[6]

So today we have the *Hardy–Weinberg* principle to recognize the two 1908 papers—scientific twins, if you like—that marked the beginning of population genetics.

QUESTIONS FOR DISCUSSION

1. Stern's proposal to credit Weinberg along with Hardy for discovering the genotype frequency formula was, in his words, "a matter of justice." But by the time that Stern made his proposal, Weinberg was already dead. Thus, like Mendel, Weinberg received credit for his discovery posthumously. We do not know if Weinberg was bothered by the fact that during his lifetime the formula was known simply as Hardy's law. However, scientists—like most other people—generally like to receive credit for their work. Can you think of other instances in which a scientist's work was recognized belatedly or posthumously? Can you think of cases in which the wrong person was credited with a scientific discovery?
2. Because his focus was on human heredity, Weinberg could not avail himself of the experimental techniques possible with plants and animals. Instead, he chose a "population approach" to the questions that interested him. How is the population approach relevant to issues in genetics today?

Stern, C. 1943. The Hardy–Weinberg law. *Science* 97:137–138.
Ibid., p. 138.

[6] Ibid.

he equilibrium frequency of heterozygotes in the population is quite low; thus, drift dominates over mutation in small populaions. For N equal to $1/u$, the reciprocal of the mutation rate, he equilibrium frequency of heterozygotes is 0.8, and for even greater values of N, the frequency of heterozygotes increases asymptotically toward 1. Thus, in large populations, mutation dominates over drift; every mutational event creates a new allele, and each new allele contributes to the heterozygosity because the large size of the population protects the allele from being lost by random genetic drift.

Values of $\hat{H}$ in natural populations vary among species. In the African cheetah, for example, $\hat{H}$ is 1 percent or less among a sample of loci, suggesting that over evolutionary time, population size in this species has been small. In humans, $\hat{H}$ is estimated to be about 12 percent, suggesting that over evolutionary time population size has averaged about 30,000 to 40,000 individuals. Estimates of population size that are derived from heterozygosity data are typically much smaller than estimates obtained from census data. The reason for this discrepancy is that the estimates based on heterozygosity data are *genetically effective* population sizes—sizes that take into account restrictions on mating and reproduction, as well as temporal fluctuations in the number of mating individuals. The genetically effective size of a population is almost always less than the census size of a population.

KEY POINTS

- Selection involving heterozygote superiority (balancing selection) creates a dynamic equilibrium in which different alleles are retained in a population despite their being harmful in homozygotes.
- In humans sickle-cell anemia is associated with balancing selection at the locus for β-globin.
- Selection against a deleterious recessive allele that is replenished in the population by mutation leads to a dynamic equilibrium in which the frequency of the recessive allele is a simple function of the mutation rate and the selection coefficient: $q = \sqrt{u/s}$.
- A population's acquisition of selectively neutral alleles through mutation is balanced by the loss of these alleles through genetic drift. At equilibrium, the frequency of heterozygotes involving these alleles is a function of the population's size and the mutation rate: $H = 4Nu/(4Nu + 1)$.

▶ Basic Exercises

ILLUSTRATE BASIC GENETIC ANALYSIS

1. Calculate the allele frequencies from the following population data:

Genotype	Number
AA	68
Aa	42
aa	24
Total	134

Answer: The frequency of the *A* allele, *p*, is $[(2 \times 68) + 42]/(2 \times 134) = 0.664$. The frequency of the *a* allele, *q*, is $[(2 \times 24) + 42]/(2 \times 134) = 0.336$.

2. Predict the Hardy–Weinberg genotype frequencies using the allele frequencies calculated in Exercise 1. Are these frequencies in agreement with the observed frequencies?

Answer: The basic calculations are summarized in the following table:

Genotype	Obs. No.	H–W Frequency	Exp. No.	Obs. – Exp. No.
AA	68	$p^2 = 0.441$	59.1	8.9
Aa	42	$2pq = 0.446$	59.8	−17.8
aa	24	$q^2 = 0.113$	15.1	8.9

To test for agreement between the observed and expected numbers, we calculate a χ^2 test statistic with 1 degree of freedom: $\chi^2 = \Sigma(\text{Obs} - \text{Exp})^2/\text{Exp} = 12.0$, which exceeds the critical value for this test statistic. Thus, we reject the hypothesis that the genotype frequencies calculated from the Hardy–Weinberg principle agree with the observed frequencies. Evidently, the population is not in Hardy–Weinberg equilibrium.

3. In a population that has been mating randomly for many generations, two phenotypes are segregating; one is due to a dominant allele *G*, the other to a recessive allele *g*. The frequencies of the dominant and recessive phenotypes are 0.7975 and 0.2025, respectively. Estimate the frequencies of the dominant and recessive alleles.

Answer: The frequency of the dominant phenotype represents the sum of two Hardy–Weinberg genotype frequencies: p^2 (*GG*) + $2pq$ (*Gg*). The frequency of the recessive phenotype represents just one Hardy–Weinberg genotype frequency, q^2 (*gg*). To estimate the frequency of the recessive allele, we take the square root of the observed frequency of the recessive phenotype: $q = \sqrt{0.2025} = 0.45$. The frequency of the dominant allele is obtained by subtraction: $p = 1 - q = 0.55$.

4. A gene with two alleles is segregating in a population. The fitness of the recessive homozygotes is 90 percent that of the heterozygotes and the dominant homozygotes. What is the value of the selection coefficient that measures the intensity of natural selection against the recessive allele?

Answer: Using *s* to represent the selection coefficient, the fitness scheme is

Genotype	Relative Fitness
AA	1
Aa	1
aa	$1 - s$

Because the recessive homozygotes are 90 percent as fit as either of the other genotypes, the expression $1 - s = 0.9$; thus, $s = 0.1$.

5. Suppose that the alleles of the *T* gene are selectively neutral. In a population of 50 individuals, currently 34 are heterozygotes. Predict the frequency of heterozygotes in this population 10 generations in the future. Assume that the population size is constant and that mating is completely random (including the possibility of self-fertilization).

Answer: For a selectively neutral gene, evolution occurs by random genetic drift. The governing equation is $H_t = (1 - \frac{1}{2N})^t H$, where H_t is the frequency of heterozygotes *t* generations in the future, *N* is the population size, and *H* is the frequency of heterozygotes now. From the data given in the problem, $N = 50$, $H = 34/50 = 0.68$, and $t = 10$. Thus, $H_t = (0.99)^{10} \times (0.68) = 0.615$.

6. Purifying selection eliminates deleterious alleles from a population, but recurrent mutation replenishes them. Suppose that recessive lethal alleles of the *B* gene are created at the rate of 2×10^{-6} per

ıeration. What is the expected frequency of lethal alleles in a popu-on in mutation–selection equilibrium?

Answer: The frequency of lethal alleles is given by the equation $\sqrt{u/s}$ where u is the mutation rate (from dominant normal allele to recessive lethal allele) and s is the intensity of selection against the deleterious allele (in this case, $s = 1$). Thus, the expected frequency of lethal alleles in the population is $q = \sqrt{2 \times 10^{-6}} = 0.0014$.

Testing Your Knowledge

INTEGRATE DIFFERENT CONCEPTS AND TECHNIQUES

1. The A–B–O blood types of 1000 people from an isolated vil-e were determined to obtain the following data:

ɪlood Type	Number of People
A	42
B	672
AB	36
O	250

ɪimate the frequencies of the I^A, I^B, and i alleles of the A–B–O blood ɔup gene from these data.

Answer: Let's symbolize the frequencies of the I^A, I^B, and i alleles the I gene as p, q, and r, respectively, and let's assume that the geno-ɔes of this gene are in Hardy–Weinberg proportions. We begin by imating r, the frequency of the i allele. To obtain this estimate, we te that the frequency of the O blood type, which is 250/1000 = 0.25 the data, should correspond to the Hardy–Weinberg frequency of : ii genotype, r^2. Thus, if we use the Hardy–Weinberg principle in ɾerse, we can estimate the frequency of the i allele as $r = \sqrt{0.250} =$:00.

To estimate p, the frequency of the I^A allele, we note that $(p + r)^2 =$ $+ 2pr + r^2$ corresponds to the combined frequencies of the A $+ 2pr$) and O (r^2) blood types. From the data, these combined quencies are estimated to be $(42 + 250)/1000 = 0.292$. If we set $+ r)^2 = 0.292$ and take the square root, we obtain $p + r = 0.540$; :n, by subtracting r, we can estimate the frequency of the I^A allele as $= 0.540 - 0.500 = 0.040$. To estimate q, the frequency of the I^B allele, note that $p + q + r = 1$. Thus, $q = 1 - p - r = 1 - 0.040 - 0.500 =$ ɪ60.

2. A man and a woman who both have normal color vision have d three children, including a male who is color blind. The incidence color-blind males in the population from which this couple came is :0, which is unusually high for X-linked color blindness. If the color-nd male marries a female with normal color vision, what is the ance that their first child will be color blind?

Answer: Clearly, the risk that the couple will have a color-blind ild depends on the female's genotype. If the female is heterozygous · the allele for color blindness, she has a probability of 1/2 of trans-tting this allele to her first child. The male will transmit either an X romosome, which carries the mutant allele, or a Y chromosome; in her case, the female's contribution to the zygote will be determinative. To obtain the probability that the female is heterozygous for the mutant allele, we note that the incidence of color blindness among males in the population is 0.30; this number provides an estimate of the frequency of the mutant allele, q, in the population. Furthermore, because $q = 0.30$, the frequency of the wild-type allele, p, is $1 - q = 0.70$. If the genotypes in the population are in Hardy–Weinberg proportions, then the frequency of heterozygous females is $2pq = 2 \times (0.7) \times (0.3) = 0.42$. However, among females who have normal color vision, the frequency of heterozygotes is greater because homozygous mutant females have been excluded from the total. To adjust for this effect, we calculate the ratio of heterozyotes to wild-type homozygotes plus heterozygotes and specifically exclude the mutant homozygotes—that is, we compute $2pq/(p^2 + 2pq) = 2pq/[p(p + 2q)] = 2q/(p + q + q) = 2q/(1 + q)$. Substituting $q = 0.3$ into the last expression, we estimate the frequency of heterozygotes among females with normal color vision (wild-type homozygotes plus heterozygotes) to be $2 \times (0.3)/(1 + 0.3) = 0.46$. This number is the chance that the female in question is a heterozygous carrier of the mutant allele. The probability that her first child will be color blind is the chance that she is a carrier (0.46) times the chance that she will transmit the mutant allele to her child (1/2); thus, the risk for the child to be color blind is $(0.46) \times (1/2) = 0.23$.

3. The HBB^S allele responsible for sickle-cell anemia is maintained in many human populations because in heterozygous condition it confers some resistance to infection by malaria parasites; however, in homozygous condition, this allele is essentially lethal. Thus, as malaria is eradicated we might expect the HBB^S allele to disappear from human populations. If the normal allele HBB^A mutates to HBB^S at a rate of 10^{-8} per generation, what ultimate frequency would you predict for the HBB^S allele in a malaria-free world?

Answer: In a malaria-free world, the advantage of maintaining the HBB^S allele in a balanced polymorphism would disappear. HBB^S HBB^A heterozygotes would have the same fitness as Hb^AHb^A homozygotes, and HBB^SHBB^S homozygotes would continue to have very low fitness—essentially zero compared to the other two genotypes. Under these circumstances, the frequency of the HBB^S allele (q) would be determined by a balance between selection against it in homozygous condition (selection coefficient $s = 1$) and introduction into the population by mutation at rate $u = 10^{-8}$ per generation. The equilibrium frequency of the Hb^S allele would be $q = \sqrt{u/s} = 0.0001$ a thousandfold less than its current frequency in malaria-infested regions of the world.

▸ Questions and Problems

ENHANCE UNDERSTANDING AND DEVELOP ANALYTICAL SKILLS

24.1 The following data for the M-N blood types were obtained from native villages in Central and North America:

Group	Sample Size	M	MN	N
Central American	86	53	29	4
North American	278	78	61	139

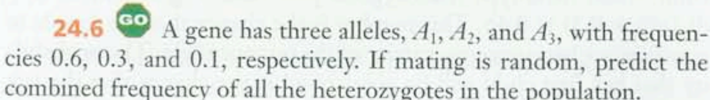
Calculate the frequencies of the L^M and L^N alleles for the two groups.

24.2 The frequency of an allele in a large randomly mating population is 0.2. What is the frequency of heterozygous carriers?

24.3 The incidence of recessive albinism is 0.0004 in a human population. If mating for this trait is random in the population, what is the frequency of the recessive allele?

24.4 In a sample from an African population, the frequencies of the L^M and L^N alleles were 0.78 and 0.22, respectively. If the population mates randomly with respect to the M-N blood types, what are the expected frequencies of the M, MN, and N phenotypes?

24.5 Human beings carrying the dominant allele *T* can taste the substance phenylthiocarbamide (PTC). In a population in which the frequency of this allele is 0.4, what is the probability that a particular taster is homozygous?



24.6 GO A gene has three alleles, A_1, A_2, and A_3, with frequencies 0.6, 0.3, and 0.1, respectively. If mating is random, predict the combined frequency of all the heterozygotes in the population.

24.7 Hemophilia is caused by an X-linked recessive allele. In a particular population, the frequency of males with hemophilia is 1/4000. What is the expected frequency of females with hemophilia?

24.8 In *Drosophila* the ruby eye phenotype is caused by a recessive, X-linked mutant allele. The wild-type eye color is red. A laboratory population of *Drosophila* is started with 25 percent ruby-eyed females, 25 percent homozygous red-eyed females, 5 percent ruby-eyed males, and 45 percent red-eyed males. (a) If this population mates randomly for one generation, what is the expected frequency of ruby-eyed males and females? (b) What is the frequency of the recessive allele in each of the sexes?

24.9 A trait determined by an X-linked dominant allele shows 100 percent penetrance and is expressed in 36 percent of the females in a population. Assuming that the population is in Hardy–Weinberg equilibrium, what proportion of the males in this population express the trait?

24.10 A phenotypically normal couple has had one normal child and a child with cystic fibrosis, an autosomal recessive disease. The incidence of cystic fibrosis in the population from which this couple came is 1/500. If their normal child eventually marries a phenotypically normal person from the same population, what is the risk that the newlyweds will produce a child with cystic fibrosis?

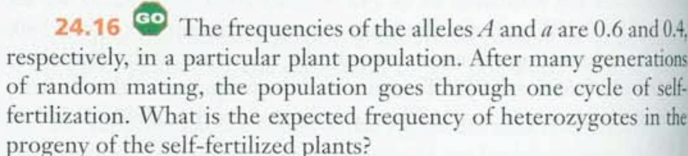
24.11 What frequencies of alleles *A* and *a* in a randomly mating population maximize the frequency of heterozygotes?

24.12 In an isolated population, the frequencies of the I^A, I^B, and *i* alleles of the A–B–O blood-type gene are, respectively, 0.15, 0.25, and 0.60. If the genotypes of the A–B–O blood type gene are in Hardy–Weinberg proportions, what fraction of the people who have type A blood in this population are expected to be homozygous for the I^A allele?

24.13 In a survey of moths collected from a natural population, a researcher found 51 dark specimens and 49 light specimens. The dark moths carry a dominant allele, and the light moths are homozygous for a recessive allele. If the population is in Hardy–Weinberg equilibrium, what is the estimated frequency of the recessive allele in the population? How many of the dark moths in the sample are likely to be homozygous for the dominant allele?

24.14 A population of Hawaiian *Drosophila* is segregating two alleles, P^1 and P^2, of the phosphoglucose isomerase (*PGI*) gene. In a sample of 100 flies from this population, 30 were P^1P^1 homozygotes, 60 were P^1P^2 heterozygotes, and 10 were P^2P^2 homozygotes. (a) What are the frequencies of the P^1 and P^2 alleles in this sample? (b) Perform a chi-square test to determine if the genotypes in the sample are in Hardy–Weinberg proportions. (c) Assuming that the sample is representative of the population, how many generations of random mating would be required to establish Hardy–Weinberg proportions in the population?

24.15 In a large population that reproduces by random mating, the frequencies of the genotypes *GG*, *Gg*, and *gg* are 0.04, 0.32, and 0.64, respectively. Assume that a change in the climate induces the population to reproduce exclusively by self-fertilization. Predict the frequencies of the genotypes in this population after many generations of self-fertilization.



24.16 GO The frequencies of the alleles *A* and *a* are 0.6 and 0.4, respectively, in a particular plant population. After many generations of random mating, the population goes through one cycle of self-fertilization. What is the expected frequency of heterozygotes in the progeny of the self-fertilized plants?

24.17 Each of two isolated populations is in Hardy–Weinberg equilibrium with the following genotype frequencies:

Genotype	*AA*	*Aa*	*aa*
Frequency in Population 1:	0.04	0.32	0.64
Frequency in Population 2:	0.64	0.32	0.04

(a) If the populations are equal in size and they merge to form a single large population, predict the allele and genotype frequencies in the large population immediately after merger.
(b) If the merged population reproduces by random mating, predict the genotype frequencies in the next generation.
(c) If the merged population continues to reproduce by random mating, will these genotype frequencies remain constant?

24.18 A population consists of 25 percent tall individuals (geno-e *TT*), 25 percent short individuals (genotype *tt*), and 50 percent ividuals of intermediate height (genotype *Tt*). Predict the ultimate enotypic and genotypic composition of the population if, genera-n after generation, mating is strictly assortative (that is, tall indi-uals mate with tall individuals, short individuals mate with short ividuals, and intermediate individuals mate with intermediate ividuals).

24.19 In controlled experiments with different genotypes of an ect, a researcher has measured the probability of survival from fer-zed eggs to mature, breeding adults. The survival probabilities of three genotypes tested are: 0.92 (for *GG*), 0.90 (for *Gg*), and 0.56 r *gg*). If all breeding adults are equally fertile, what are the relative nesses of the three genotypes? What are the selection coefficients the two least fit genotypes?

24.20 In a large randomly mating population, 0.84 of the indi-luals express the phenotype of the dominant allele *A* and 0.16 ex-ess the phenotype of the recessive allele *a*. (a) What is the frequency the dominant allele? (b) If the *aa* homozygotes are 5 percent less fit in the other two genotypes, what will the frequency of *A* be in the xt generation?

24.21 Because individuals with cystic fibrosis die before they n reproduce, the coefficient of selection against them is $s = 1$. As-me that heterozygous carriers of the recessive mutant allele respon-ole for this disease are as fit as wild-type homozygotes and that the pulation frequency of the mutant allele is 0.02. (a) Predict the inci-nce of cystic fibrosis in the population after one generation of selec-on. (b) Explain why the incidence of cystic fibrosis hardly changes en with $s = 1$.

24.22 For each set of relative fitnesses for the genotypes *AA*, *Aa*, d *aa*, explain how selection is operating. Assume that $0 < t < s < 1$.

	AA	*Aa*	*aa*
Case 1	1	1	$1-s$
Case 2	$1-s$	$1-s$	1
Case 3	1	$1-t$	$1-s$
Case 4	$1-s$	1	$1-t$

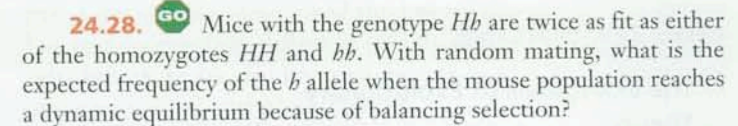
24.23 The frequency of newborn infants homozygous for a recessive lethal allele is about 1 in 25,000. What is the expected frequency of carriers of this allele in the population?

24.24 A population of size 50 reproduces in such a way that the population size remains constant. If mating is random, how rapidly will genetic variability, as measured by the frequency of heterozygotes, be lost from this population?

24.25 A population is segregating three alleles, A_1, A_2, and A_3, with frequencies 0.2, 0.5, and 0.3, respectively. If these alleles are selectively neutral, what is the probability that A_2 will ultimately be fixed by genetic drift? What is the probability that A_3 will ultimately be lost by genetic drift?

24.26 A small island population of mice consists of roughly equal numbers of males and females. The Y chromosome in one-fourth of the males is twice as long as the Y chromosome in the other males because of an expansion of heterochromatin. If mice with the large Y chromosome have the same fitness as mice with the small Y chromosome, what is the probability that the large Y chromosome will ultimately be fixed in the mouse population?

24.27 In some regions of West Africa, the frequency of the HBB^S allele is 0.2. If this frequency is the result of a dynamic equilibrium due to the superior fitness of HBB^SHBB^A heterozygotes, and if HBB^SHBB^S homozygotes are essentially lethal, what is the intensity of selection against the HBB^AHBB^A homozygotes?



24.28. GO Mice with the genotype *Hh* are twice as fit as either of the homozygotes *HH* and *hh*. With random mating, what is the expected frequency of the *h* allele when the mouse population reaches a dynamic equilibrium because of balancing selection?

24.29 A completely recessive allele *g* is lethal in homozygous condition. If the dominant allele *G* mutates to *g* at a rate of 10^{-6} per generation, what is the expected frequency of the lethal allele when the population reaches mutation–selection equilibrium?

24.30 Individuals with the genotype *bb* are 20 percent less fit than individuals with the genotypes *BB* or *Bb*. If *B* mutates to *b* at a rate of 10^{-6} per generation, what is the expected frequency of the allele *b* when the population reaches mutation–selection equilibrium?

Genomics on the Web

at http://www.ncbi.nlm.nih.gov/

he mutant allele that causes sickle-cell anemia is prevalent in areas here people have a high probability of contracting malaria, which is aused by a parasite transmitted by mosquitoes. Click on the links for Malaria and Mosquito on the Genomic biology page to find informa-on on the malaria parasite *Plasmodium falciparum* and on the mos-uito vector *Anopheles gambiae*.

1. How large is the *Plasmodium* genome? How many chromo-omes does it comprise? How large is the *Anopheles* genome? How many chromosomes does it comprise? Have the genomes of these organisms been sequenced completely?

2. On the *Plasmodium* web page, click on the overview link to bring up a page with summary information on this parasite. Under related resources, click on WHO/Malaria info to bring up a page with links to information about various aspects of malaria. How widespread is the disease? How is it being treated today? How is the *Plasmodium* parasite transmitted from one person to another?

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When Science Takes the Witness Stand

In courts of law, forensic testimony often goes unchallenged by a scientifically naive legal community. Forensic methods must be screened with greater care if justice is to be served

by Peter J. Neufeld and Neville Colman

In the early evening of November 21, 1974, powerful bombs ripped through two pubs in the industrial city of Birmingham, England, leaving 21 dead and 162 injured. The government immediately blamed the Irish Republican Army for the attacks and mounted a massive search for the perpetrators. After a railroad clerk reported that six Irishmen had boarded a train in Birmingham minutes before the first bomb blast, police intercepted the men as they disembarked at the port of Heysham. The six men were taken to the police station, and there, their hands were swabbed with chemicals that would reveal the presence of any nitrites, which would be consistent with the recent handling of explosives. The forensic scientist who performed this procedure, known as the Greiss test, reported positive findings on the right hands of two of the six suspects. That evidence became the linchpin of the government's successful prosecution of the "Birmingham Six."

Now, 16 years later, the six men may be released. The Greiss test, on which their convictions had been largely based, has proved unreliable. It turns out that a variety of common substances such as old playing cards, cigarette packages, lacquer and aerosol spray will, along with explosives, yield a positive result. As it happened, the six men had spent most of their train ride to Heysham playing cards and smoking cigarettes.

The Birmingham case raises troubling issues about the application of forensic technology to criminal investigations. Since the discovery of fingerprinting at the turn of this century, science has assumed an increasingly powerful role in the execution of justice. Indeed, scientific testimony is often the deciding factor for the judicial resolution of civil and criminal cases. The scientific analysis of fingerprints, blood, semen, shreds of clothing, hair, weapons, tire treads and other physical evidence left at the scene of a crime can seem more compelling to a jury than the testimony of eyewitnesses. As one juror put it after a recent trial in Queens, N.Y., "You can't argue with science."

Scientists generally welcome this trend. Because the scientific community polices scientific research, subjecting new theories and findings to peer review and independent verification, it is often assumed the same standards prevail when science is applied to the fact-finding process in a judicial trial. But in reality such controls are absent in a court of law. Instead nonscientists—lawyers, judges and jurors—are called on to evaluate critically the competence of a scientific witness. Frequently lawyers are oblivious of potential flaws in a scientific method or argument and so fail to challenge it. At other times, the adversaries in a case will present opposing expert opinions, leaving it up to a jury of laypersons to decide the merits of the scientific arguments.

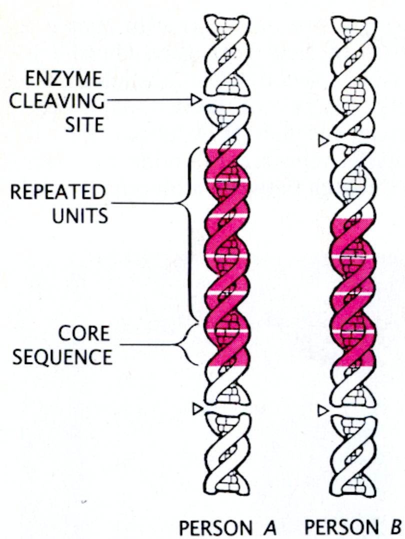
The disjunction between scientific and judicial standards of evidence has allowed novel forensic methods to be used in criminal trials prematurely or without verification. The problem has become painfully apparent in the case of forensic DNA profiling, a recent technique that in theory can identify an individual from his or her DNA with a high degree of certainty. Although

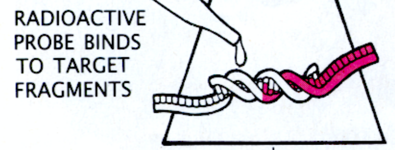
PETER J. NEUFELD and NEVILLE COLMAN have collaborated for several years on the problem of admitting new scientific techniques into criminal cases and have lectured on the subject to both defense attorneys and prosecutors. Neufeld, an attorney specializing in criminal defense and civil-rights litigation, was co-counsel in *People v. Castro,* in which DNA evidence was first successfully challenged. He is a member of the New York State governor's panel on forensic DNA analysis. Neufeld received his J.D. in 1975 from the New York University School of Law and is adjunct associate professor at the Fordham University School of Law. Colman is director of the Center for Clinical Laboratories at Mount Sinai Medical Center in New York City. He received his M.D. in 1969 and his Ph.D. in 1974 from the University of the Witwatersrand, Johannesburg. He has advised counsel and testified in legal proceedings involving the admissibility of scientific evidence.

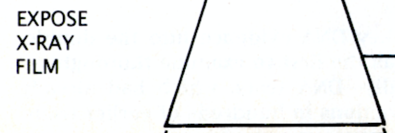
many aspects of forensic DNA identification have not been adequately examined by the scientific community, police and prosecutors have carried out DNA analysis in more than 1,000 criminal investigations in the U.S. since 1987. Few of these cases reached trial. In most instances, defendants pleaded guilty on advice of counsel after a presumably infallible DNA test declared a match.

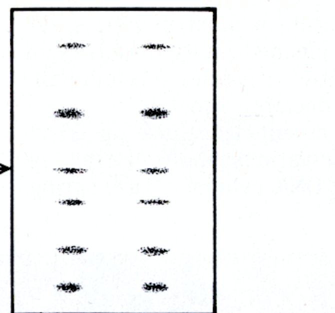
Several recent cases have raised serious reservations about the claims made for DNA evidence. Last spring, during a pretrial hearing in *People v. Castro* in New York City, Michael L. Baird of Lifecodes Corporation in Valhalla, N.Y., one of the two major commercial forensic DNA laboratories in the U.S., reported the odds of a random match between a bloodstain and

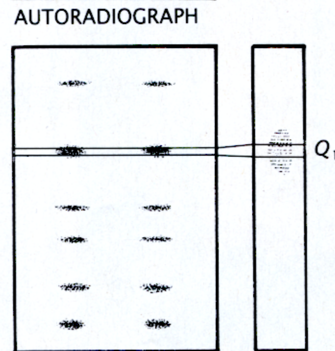
EXPERT WITNESS Lorraine Flaherty, a molecular geneticist at the New York State Department of Health, testifies on DNA analysis during last year's pretrial hearing of *People v. Castro.* Bronx County Supreme Court Justice Gerald Sheindlin later ruled against admitting key DNA evidence into the double-murder trial. The case was the first to examine thoroughly—and challenge successfully—DNA tests, which had already been used to obtain convictions in hundreds of earlier trials.

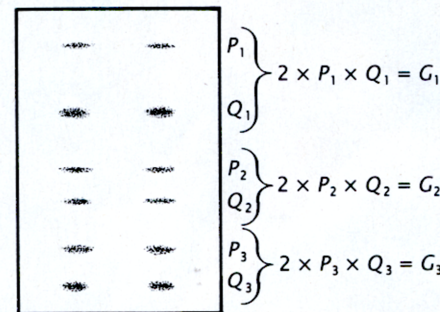












DNA IDENTIFICATION currently hinges on the existence of certain regions in DNA, called restriction fragment length polymorphisms (RFLP's), which contain "core" sequences (*color*) that are repeated in tandem a variable number of times from person to person. Each RFLP can be identified by a special probe that recognizes and binds to any fragment containing the core sequence. Special enzymes snip RFLP's out of DNA. Forensic casework involves taking DNA extracted from evidence and from, for example, a suspect's blood, breaking it up into RFLP's and separating them by gel electrophoresis. A radioactive probe binds to the RFLP's, whose positions are then recorded as dark bands on X-ray film. If the striped patterns from the evidence and from the suspect appear to match, one then calculates the probability of such a match occurring by chance.

the suspect at one in 100 million. Eric S. Lander of Harvard University and the Massachusetts Institute of Technology examined the same data and arrived at odds of one in 24. Ultimately, several proponents of DNA testing denounced Lifecodes' data in the case as scientifically unreliable. Some of Lifecodes' key methods were repudiated, casting doubt on the integrity of hundreds of earlier criminal convictions. The ongoing debate over DNA testing underscores the need to deal more effectively with the difficulties that arise whenever complex scientific technology is introduced as evidence in a court of law.

A trial is ideally a search for truth. To help juries in their quest, the law allows qualified experts to testify and express opinions on matters in which they are professionally trained. Yet the esoteric nature of an expert's opinions, together with the jargon and the expert's scholarly credentials, may cast an aura of infallibility over his or her testimony. Hence, to prevent juries from being influenced by questionable evidence or expert testimony, U.S. courts usually review the material in a pretrial hearing or outside the presence of the jury.

To be admitted as evidence, a forensic test should, as a matter of common sense, satisfy three criteria: the underlying scientific theory must be considered valid by the scientific community; the technique itself must be known to be reliable; and the technique must be shown to have been properly applied in the particular case.

The expression of common sense in a court of law, however, is at times elusive. A majority of U.S. courts decide on the admissibility of scientific evidence based on guidelines established in 1923 by *Frye v. U.S.*, in which the Court of Appeals for the District of Columbia affirmed a lower court's decision to exclude evidence derived from a precursor of the polygraph. "Just when a scientific principle or discovery crosses the line between the experimental and demonstrable stages is difficult to define," the court declared in *Frye*. "Somewhere in this twilight zone the evidential force of the principle must be recognized, and while courts will go a long way in admitting expert testimony deduced from a well-recognized scientific principle or discovery, the thing from which the deduction is made must be sufficiently established to have gained general acceptance in the particular field in which it belongs."

Judges, scientists, lawyers and legal

scholars have all criticized the *Frye* standard. Some say it is too vague. Some argue that it is unduly restrictive. Still others complain that it is not restrictive enough. Should "general acceptance," for example, require a consensus or a simple majority of scientists? Also, what is it that must be generally accepted? In the case of DNA profiling, is it the theory that no two individuals, except for identical twins, have the same DNA? Is it the various techniques employed in the test, such as Southern blotting and gel electrophoresis? Or is it the specific application of DNA profiling to dried blood and semen samples recovered from the scene of a crime?

Furthermore, what is the appropriate "particular field" in which a technique must be accepted? Does a test for DNA profiling have to be accepted only by forensic serologists, or must it also be recognized by the broader community of human geneticists, hematologists and biochemists? In a recent California case, DNA evidence analyzed by means of the polymerase chain reaction (PCR) was excluded because that method was not generally accepted by forensic scientists. Yet several months earlier a Texas court that was evaluating the identical PCR method looked more broadly to the opinions of molecular biologists and human geneticists and reached the opposite conclusion.

For many applications of science to forensics, the underlying theory is well established, and legal debate rages mainly over whether one must prove only that a technique is generally accepted for scientific research or, more strictly, that the technique is reliable when applied to forensics.

Why the distinction between nonforensic and forensic applications? Scientists commonly accept that when any technology is tried in a different application, such as forensics, it must be tested thoroughly to ensure an empirical understanding of the technique's usefulness and limitations. Indeed, many a technique that has proved reliable for research—polygraphy, for example—has turned out to be of questionable reliability when applied to forensic casework.

Clearly, in order for the courts to evaluate forensic evidence, judges and lawyers must be able to appreciate the scientific issues at hand. Regrettably, lawyers rarely do more than review the qualifications of the expert (typically based on perfunctory queries about institutional affiliation and publications) and verify the facts on which the expert's conclusions are based. The reason for this limited inquiry is simple: most lawyers and judges lack the adequate scientific background to argue or decide the admissibility of expert testimony. Often judges think—mistakenly, in our opinion—that justice is best served by admitting expert testimony into evidence and deferring to the jury for the determination of its weight.

The problem of scientific illiteracy is compounded by the tendency of judges to refuse to reconsider the validity of a particular kind of scientific evidence once it has been accepted by another judge in an earlier case. This practice is founded on the well-recognized need to respect precedent in order to ensure the uniform administration of justice. But in the case of forensic tests, the frequent failure of courts to take a fresh look at the underlying science has been responsible for many a miscarriage of justice.

Perhaps the most notorious example of the problem is the so-called paraffin test (a cousin of the Greiss test employed in the Birmingham Six investigation), which was used by crime laboratories throughout the U.S. to detect nitrite and nitrate residues, presumably from gunpowder, on suspects' hands to show that they had recently fired a gun. The test was first admitted as scientific evidence in a 1936 trial in Pennsylvania. Other states then simply adopted that decision without independently scrutinizing the research.

For the next 25 years innumerable people were convicted with the help of this test. It was not until the mid-1960's that a comprehensive scientific study revealed damning flaws in the paraffin test. In particular, the test gave an unacceptably high number of false positives: substances other than gunpowder that gave a positive reading included urine, tobacco, tobacco ash, fertilizer and colored fingernail polish. In this instance the legal process failed, allowing people accused of crimes to be convicted on evidence that later proved to be worthless.

More recently the debate over scientific courtroom evidence has centered on two applications of biotechnology: protein-marker analysis and DNA identification. Both techniques employ gel electrophoresis to reveal genetic differences, called polymorphisms, in blood proteins and DNA. These two techniques can potentially match blood, semen or other such evidence found at a crime scene to a suspect or victim.

In the late 1960's crime laboratories became interested in protein polymorphisms in populations. The techniques for studying protein polymorphisms were originally developed as tools for population geneticists and were experimentally tested, published in refereed journals and independently verified. The techniques were then modified by and for law-enforcement personnel in order to cope with problems unique to forensic samples, such as their often limited quantity, their unknown age and the presence of unidentified contaminants. These modifications were rarely published in the scientific literature or validated by independent workers.

For example, molecular geneticists study polymorphic proteins in red blood cells and serum by using fresh, liquid blood and analyzing it under controlled laboratory conditions, all subject to scientific peer review. These techniques were then adapted for use on forensic samples of dried blood by the introduction of various modifications, few of which were subjected to comparable scientific scrutiny. No one ever adequately explored the effects of environmental insults to samples, such as heat, humidity, temperature and light. Neither did anyone verify the claim that forensic samples would not be affected significantly by microbes and unknown substances typically found on streets or in carpets.

One of the major modifications made by forensic laboratories was the "multisystem" test. In the original version of this test, three different polymorphic proteins were identified in a single procedure; the purpose was to derive as much information as possible from a small sample. The three-marker multisystem test was further modified by the addition of a fourth protein marker in 1980 by the New York City Medical Examiner's serology laboratory.

By 1987 evidence derived from the "four-in-one" multisystem had been introduced in several hundred criminal prosecutions in New York State. In that year, however, during a pretrial hearing in *People v. Seda,* the director of the New York City laboratory admitted under cross-examination that only one article had been published about that system—and that the article had recommended the test be used only to screen out obvious mismatches because of a flaw that tended to obscure the results.

In *People v. Seda,* the judge ruled that the four-in-one multisystem did not satisfy the *Frye* standard of general acceptance by the scientific commu-

nity and so could not be introduced into evidence. Unfortunately, *Seda* was the first case involving the test in which the defense went to the effort of calling witnesses to challenge the technology. Consequently, the integrity of hundreds of earlier convictions stands in doubt.

In the past two years DNA profiling has all but eclipsed protein markers in forensic identification. The technique is based on a method originally developed to study the inheritance of diseases, both to identify the disease-causing genes in families known to harbor an inherited disease and to predict individual susceptibility when the gene is known.

Crime investigators have embraced the new technique because it offers two significant advantages over conventional protein markers. First, DNA typing can be conducted on much smaller and older samples. And second, DNA typing was reported to offer from three to 10 orders of magnitude greater certainty of a match. Promotional literature distributed by Lifecodes asserts that its test "has the power to identify one individual in the world's population." Not to be outdone, Cellmark Diagnostics in Germantown, Md.—Lifecodes' main competitor—claims that with its method, "the chance that any two people will have the same DNA print is one in 30 billion." Yet, as testimony in the *Castro* case showed, such claims can be dubious.

The hype over DNA typing spreads the impression that a DNA profile identifies the "genetic code" unique to an individual and indeed is as unique as a fingerprint. Actually, because 99 percent of the three billion base pairs in human DNA are identical among all individuals, forensic scientists look for ways to isolate the relatively few variable regions. These regions can be cut out of DNA by restriction enzymes and are called restriction fragment length polymorphisms (RFLP's).

For DNA identification, one wants RFLP's that are highly polymorphic—that is, those that have the greatest number of variants, or alleles, in the population. It turns out that certain regions of human DNA contain "core" sequences that are repeated in tandem, like freight cars of a train. The number of these repeated sequences tends to vary considerably from person to person; one person might have 13 repeated units at that locus, whereas another might have 29. Special restriction enzymes cut DNA into millions of pieces, including fragments that contain the repeated segments. Because the number of repeated segments varies among individuals, so too does the overall length of these fragments vary.

How can these variable fragments be picked out of the haystack of irrelevant DNA segments? The answer lies in "probes" that bind only to fragments containing the core sequence. If the core sequence occurs at only one DNA locus, the probe is called a single-locus probe. If the core sequence occurs at many different loci, the probe is called a multilocus probe. Forensic laboratories currently make use of three different methods of DNA typing: single-locus RFLP, multilocus RFLP and the polymerase chain reaction. Because the single-locus system is the one most widely employed in forensic DNA identification, we will describe it in some detail.

For forensic DNA identification by single-locus RFLP analysis, DNA from various sources is digested with restriction enzymes, placed in separate lanes on an electrophoretic gel and subjected to an electric field. The field pulls fragments down the lane, with smaller fragments traveling faster than larger ones. The fragments, now sorted by size, are denatured into single strands and transferred from the gel onto a nitrocellulose or nylon membrane, which fixes the fragments in place. (Incidentally, anyone who handles nitrocellulose might test positive on the Greiss test!)

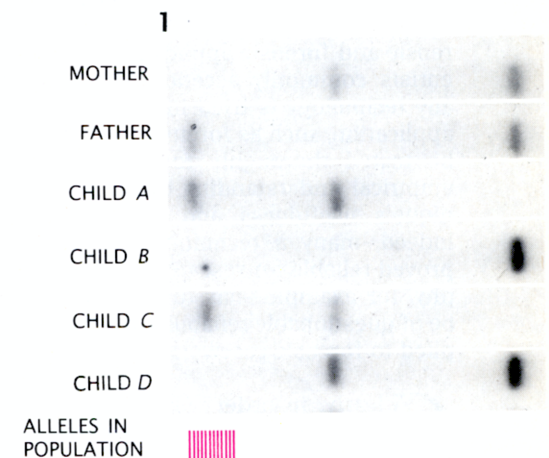
At this point, a radioactive probe is applied, which hybridizes, or binds, to the polymorphic fragments. The mesh is then laid on a sheet of X-ray film to produce an autoradiograph. The radioactively labeled fragments are thereby revealed as a series of bands resembling a railroad track with irregularly spaced ties; the position of the bands is a measure of the size of the polymorphic fragments. The probe can be rinsed away, and a new probe can be applied to identify a different set of alleles.

The autoradiograph resulting from a single-locus probe will ordinarily show alleles of two distinct sizes, one inherited from each parent; such a pattern indicates that the person is heterozygous for that locus. If the probe reveals only one distinct allele, it is assumed that the person inherited the same-size allele from both parents and that the person is homozygous for the locus. Forensic DNA-testing laboratories typically employ several single-locus probes, each of which binds to a different site.

To determine whether two samples of DNA come from a single source, one examines the bands identified by a particular probe on the autoradiograph and decides whether they match. One then refers to data from population-genetics studies to find out how often that particular allele size occurs. A typical allele might be found in 10 percent of the population, making it not all that unlikely that two random people will carry the same allele. But if one looks at alleles at three or four different sites, it becomes increasingly unlikely that two individuals will have the same alleles for all the sites. It is this hypothesis that gives DNA profiling its persuasive power.

How well does forensic DNA profiling stand up under the *Frye* standard? Certainly the underlying theory—that no two people, except for identical twins, have the identical DNA—is unquestioned, and so DNA identification is possible in theory. But is that theory being applied to give a reliable forensic test? And if so, is that test being carried out properly?

In scientific and medical research, DNA typing is most often employed to trace the inheritance of disease-causing alleles within a family. In this diagnostic application, however, one can assume that one allele was inherited from the mother and the other from the father. Because each parent has only two alleles for that gene, barring a mutation, the pattern observed in the child is limited at most to four possible combinations. In addition, if the results are ambiguous, one can rerun the experiment with fresh blood sam-



FORENSIC DNA TYPING is fraught with uncertainty. If the autoradiographs in group 1 are assumed to be from one family, then the alleles of the children must be derived from the parents, even though one of the bands for child *C* is visibly

ples or refer to the alleles of other family members.

In forensic DNA typing, however, it is much more difficult to determine whether an allele from one sample is identical to an allele from another. In the RFLP systems employed in forensics, the number of alleles can run into the hundreds—in contrast to the four from which one must choose when identifying the alleles of a child whose parents are known. Indeed, forensic RFLP systems produce so many different alleles that they virtually form a continuum. In some RFLP's the most common alleles can be crowded into a quarter-inch span on a 13-inch lane. Gel electrophoresis can resolve only a limited number of alleles, however—perhaps between 30 and 100 depending on the particular RFLP—and so alleles that are similar, but not the same, in size may be declared identical. Hence, it can become difficult indeed to declare with confidence that one band matches another. What is worse, forensic samples are often limited in amount and so cannot be retested if ambiguities arise.

These inherent difficulties are further complicated by a problem called band shifting. This phenomenon occurs when DNA fragments migrate at different speeds through separate lanes on a single gel. It has been attributed to a number of factors, involving variables such as the preparation of gels, the concentrations of sample DNA, the amount of salt in the DNA solution and contamination. Band shifting can occur even if the various lanes contain DNA from the same person. Because allele sizes in forensic RFLP systems are closely spaced, it is difficult to know whether the relative positions of bands arise purely from the size of allele fragments or whether band shifting might play a part.

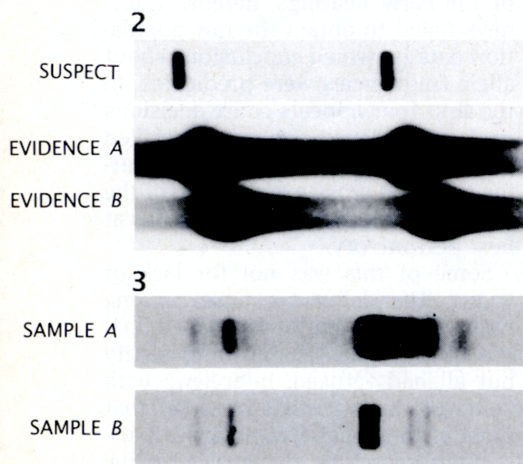
The courts' handling of band shifting is an excellent illustration of the problems that arise when courts, rather than the scientific peer-review process, take on the task of determining whether a method is reliable. Two years ago, when DNA evidence was first introduced in U.S. courtrooms, most forensic DNA scientists rejected the existence of band shifting. But now some experts think band shifting occurs in perhaps 30 percent of forensic DNA tests. There are now many theories about the cause, but as of this writing not one refereed article on the subject has been published.

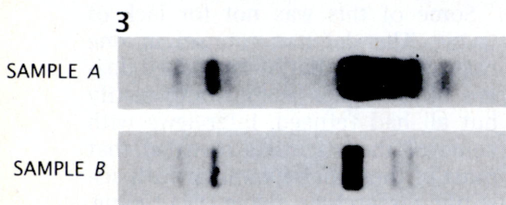
Forensic DNA laboratories are rushing to develop special probes that bind to monomorphic loci—restriction-enzyme fragments that are the same size in every person—as a possible way to control for band shifting. In theory, if the monomorphic regions are displaced, one would know that band shifting had occurred and could then calculate a correction factor. The difficulty again is that neither this method, nor any other possible solution, has been peer reviewed.

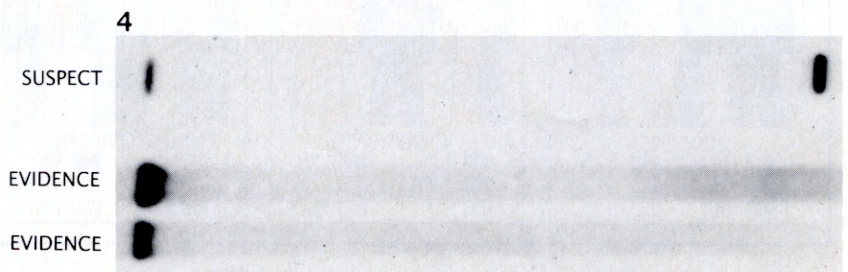
Yet in a rape case tried last December in Maine, *State v. McLeod,* the laboratory director who had supervised the DNA tests for the prosecution testified that a correction factor derived from a monomorphic probe allowed him to declare a match between the suspect's blood and the semen recovered from the victim, even though the bands were visibly shifted. When evidence then came to light that a second monomorphic probe indicated a smaller correction factor, which did not account for the disparity between the bands, he acknowledged that monomorphic probes may yield inconsistent correction factors; nevertheless, he argued that the first correction was appropriate to the bands in question. The prosecutor, though, recognized the folly of defending this argument in the absence of published supporting data and withdrew the DNA evidence. In dozens of other cases, however, judges have been persuaded by the same types of arguments, even though there is no body of research to guide the court. As a matter of common sense, the proper place to first address such issues is in scientific journals, not the courtroom.

Another major problem that arises in forensic DNA typing is contamination. More often than not, crime-scene specimens are contaminated or degraded. The presence of bacteria, organic material or degradation raises the risk of both false positives and false negatives. For example, contamination can degrade DNA so that the larger fragments are destroyed. In such instances a probe that should yield two bands may yield only one (the smaller band).

Research laboratories employ internal controls to avoid the misinterpretation that can result from such artifacts. But such controls may not be suitable for forensic casework. For example, one suggested control for band shifting is to run a mixing experiment: sample *A* is run in lane one, sample *B* in lane two and *A* and *B* in lane three. If







shifted. But if that same lane were of a person whose parentage is unknown, then the band could correspond to one of the other alleles (*color bands*) observed in the population. In group 2, the band patterns from the suspect and from evidence *A* and *B* appear to be displaced relative to one another, which may indicate a band shift. In group 3, sample *A* contains all of the bands from sample *B*, along with extra bands, possibly from contaminants. In group 4, a suspect has two bands, whereas the forensic evidence has only one; the "missing" band may have resulted because degradation of the DNA destroyed the larger fragments. On the other hand, all of these cases could also indicate a real genetic difference.

both samples are from the same person, then ideally lane three would produce one set of bands, whereas if they are from different people, it would show two sets of bands. Unfortunately, in forensic casework there is often not enough material to run a mixing experiment. What is more, recent unpublished studies indicate that certain contaminants, such as dyes, can bind to DNA and alter its mobility in a gel, so that a mixing experiment using samples from the same person can produce two sets of bands.

The power of forensic DNA typing arises from its ability not only to demonstrate that two samples exhibit the same pattern but also to suggest that the pattern is extremely rare. The validity of the data and assumptions on which forensic laboratories have been relying to estimate the rarity are currently being debated within the scientific community.

There are two particularly important criticisms. First, because it is difficult to discriminate accurately among the dozens of alleles at a particular locus, the task of calculating the frequency with which each allele appears in the population is inherently compromised. Second, the statistical equations for calculating the frequency of a particular pattern of alleles apply only to a population that has resulted from random mating—a condition that is called Hardy-Weinberg equilibrium.

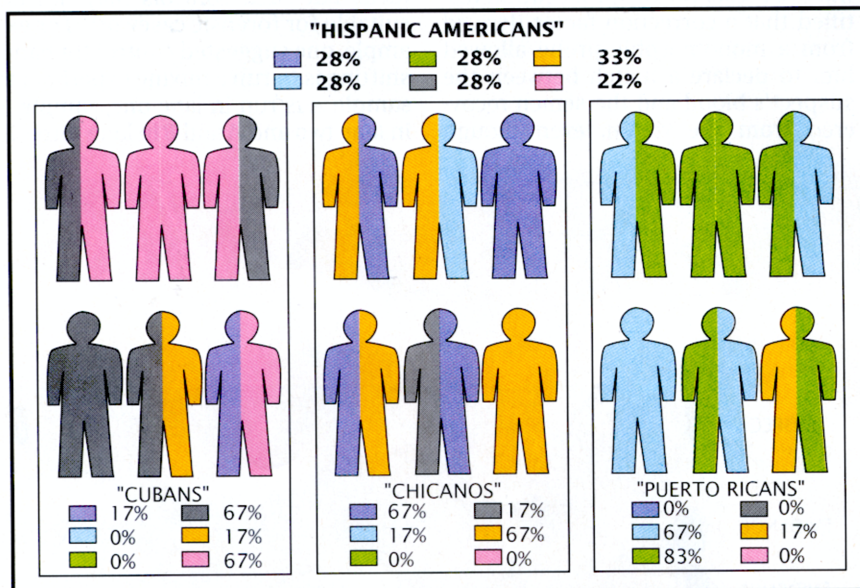
If a population is in Hardy-Weinberg equilibrium, one can assume allele types are shuffled at random. The occurrence of one allele is then independent of the occurrence of a second allele. One can therefore calculate the frequency of the "genotype," or a particular pair of alleles, for a specific locus by multiplying the frequency of each allele and doubling it (because one has the same probability of inheriting each allele from both parents). The frequency of a genotype for a combination of loci is then obtained simply by multiplying the frequency of the genotype for each individual locus. For example, if the genotypes at loci *A*, *B*, *C* and *D* each occur in 10 percent of the population, then the probability that a person would have these genotypes at all four loci is .1 multiplied by itself four times: .0001.

Forensic DNA laboratories carry out these calculations based on data they have assembled themselves. Most of the data have not been published in peer-review journals or independently validated. One problem is that none of the major laboratories employs the same RFLP system. And even if the laboratories decide to adopt uniform probes and enzymes, the results may still differ significantly unless they all also adopt identical protocols. Commercial DNA-testing laboratories are reluctant to do so, however, because each considers its RFLP system to be proprietary, and the probes and enzymes are sold or licensed to crime laboratories around the country.

Another serious issue is that some populations may not be in equilibrium, in which case neither the alleles nor the various loci may be independent. For such a population, there is as yet no consensus on how to calculate the frequency of a genotype (given the limited data bases of the forensic DNA laboratories). As matters stand, population geneticists are debating whether various racial and ethnic communities exhibit significant population substructures so as to preclude the use of current data bases for the highly polymorphic systems employed in forensic DNA identification. For example, do Hispanics in the U.S. constitute a single mixed population? Or is there nonrandom mating, with Cubans more likely to mate with other Cubans and Chicanos more likely to mate with other Chicanos? Should there be a separate data base on allele frequencies within each of these subpopulations? To find out, population geneticists will need to gather more data.

More than 1,000 criminal investigations in the U.S. have now involved DNA evidence, but in only a few dozen cases has DNA evidence been challenged in a pretrial hearing. According to our own study of these hearings, until the *Castro* case in New York, not one of these hearings addressed the problems of forensic DNA typing that distinguish it from diagnostic DNA typing. In all but two of the early hearings, defense attorneys failed to obtain the raw population data on which conclusions about allele frequencies were predicated. In the first four appeals-court decisions on DNA evidence, the defense failed to present any expert witnesses during trial, and cross-examination of the prosecution's expert witnesses was at best perfunctory.

Some of this was not for lack of trying. The defense counsel in one case explained that he had asked dozens of molecular biologists to testify but all had refused. Interviews with some of the scientists revealed that most of them, being familiar with scientific research involving DNA typing, assumed the forensic application of the technique would be equally reliable. Some who were aware of possible problems were reluctant to criticize the technology publicly for fear that this would be misconstrued as a gen-



POPULATION DATA may not yet be reliable enough to calculate the frequency of a genotype accurately. In the hypothetical Hispanic-American population depicted here, a particular DNA site has six distinct alleles, each represented by its own color. Heterozygous individuals are shaded with two colors to represent the two alleles inherited from the parents; homozygous individuals, who have inherited the same allele from both parents, are shaded with one color. Allele frequencies for the entire population differ markedly from allele frequencies for the subgroups shown here.

eral attack on the underlying science.

Another troubling fact is that defense attorneys are often not able to spend the time or funds required to deal with the complexities of the issues. Novel scientific evidence is most often used to solve violent crimes, and defendants in such cases come predominantly from the less affluent sectors of society. Consequently, most of them must rely on court-appointed counsel selected from public-defender offices, legal-aid societies or the financially less successful members of the private bar. Many of these advocates are exceptionally skillful, but they often lack the time and resources to mount a serious challenge to scientific evidence. And frankly, there are also many less-than-adequate attorneys who are simply overwhelmed by the complexity of the subject.

What is more, in most states a court-appointed lawyer may not retain an expert witness without the approval of the trial judge. In recent DNA cases in Oklahoma and Alabama, for example, the defense did not retain any experts, because the presiding judge had refused to authorize funds. In the *Castro* case, a critical factor in the defense's successful challenge was the participation of several leading scientific experts—most of whom agreed to testify without a fee.

Because defendants are seldom able to challenge novel scientific evidence, we feel that independent overseeing of forensic methods is the only way to ensure justice. Specifically, national standards must be set before a scientific technique can be transferred from the research laboratory to the courtroom, and there must be laws to ensure that these standards are enforced.

The regulation of forensic laboratories has an excellent model: the Clinical Laboratories Improvement Act of 1967 (which was amended in 1988). The act established a system of accreditation and proficiency testing for clinical laboratories that service the medical profession. The law was enacted to ensure that such service laboratories, which are not subject to the same peer scrutiny as research laboratories, would nonetheless provide reliable products and services.

In contrast, no private or public crime laboratory today is regulated by any government agency. Nor is there any mandatory accreditation of forensic laboratories or requirement that they submit to independent proficiency testing. It is also troubling that there are no formally enforced, objective criteria for interpreting forensic data. Four fifths of the forensic laboratories in North America are within police or prosecutor agencies, and so there is an enormous potential for bias because technicians may be aware of the facts of the case. In short, there is more regulation of clinical laboratories that determine whether one has mononucleosis than there is of forensic laboratories able to produce DNA test results that can help send a person to the electric chair.

Accreditation and proficiency testing will work only if implemented with care. National standards for forensic testing must serve the interests of justice, not of parties who have vested interests in the technology. This is not an imaginary danger: from 1988 to 1989 a committee of the American Association of Blood Banks set out to develop national standards for forensic DNA typing and brought in two scientists to provide expertise in molecular genetics; these two happened to be the senior scientists at Lifecodes and Cellmark, the two companies that perform virtually all commercial forensic DNA identification in the U.S.

Some observers suggest delegating the task of setting national standards for forensic DNA identification to the Federal Bureau of Investigation. But there is reason to be wary of this approach. Last year the FBI began to perform forensic DNA identification without first publishing its methodology in refereed journals. In the few pretrial hearings that have challenged DNA tests conducted by the FBI, the bureau has been reluctant to supply the raw data on which it based its criteria, citing its "privilege against self-criticism"—a concept that, incidentally, has little precedent in law. The FBI also opposes independent proficiency testing, arguing that no outsider is qualified to evaluate the bureau's performance. In addition, at a recent FBI-sponsored symposium on DNA typing that attracted 300 forensic scientists from around the country, FBI personnel were alone in opposing proposals requiring laboratories to explain in writing the basis for their conclusions and to have their reports signed by the scientists and technicians who conducted the test.

The FBI's stance on these issues flies against norms established elsewhere in the scientific community. For example, if the author of a scientific article refused to divulge his or her raw data to peer review, the article would be rejected. There is also a clear consensus in favor of independent proficiency tests. If a clinical laboratory refused to comply with any reasonable public request to examine the results of proficiency tests, it would risk losing its accreditation. And it would be unthinkable for a diagnostic laboratory to deliver to the obstetrician of a pregnant woman an unsigned report with only the word "abort" appearing on the page.

Independent scientists are finally beginning to awaken to the urgency of these issues. Last fall the New York State Forensic DNA Analysis Panel proposed detailed requirements for certifying, licensing and accrediting forensic DNA laboratories. The Congressional Office of Technology Assessment is expected to issue a report on the regulation of DNA typing by the time this article appears. The National Academy of Sciences has appointed a committee to study appropriate standards for DNA typing and is expected to issue a report early next year.

It is regrettable that these measures were set in motion only after flaws in current DNA typing came to light in the courtroom. We hope the anticipated reforms will enhance the interests of justice in the future, although this may be small solace to defendants who were wrongfully convicted or to crime victims who saw the true culprit set free. It is our hope that, with appropriate national standards and regulation of forensic laboratories, powerful new forensic techniques such as DNA typing will serve an important and beneficial role in criminal justice. When all is said and done, there should be no better test for identifying a criminal—or for exonerating an innocent suspect.

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DNA goes to court

Caitlin Smith, Stephen Strauss & Laura DeFrancesco

DNA profiling is playing a growing role in solving crimes, identifying victims of natural and unnatural disasters and even tracking diplomats. Some forensic experts are looking to advances in genome technologies to gain further ground against criminals.

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DNA forensics has not been a field where innovation proceeds by leaps and bounds. Profiles of individuals in forensic databases worldwide are based on a standard set of 13 short tandem repeats (STRs) in human genomes that have been in use for over two decades. Recently, DNA assays for eye color determination have also been added to law enforcement's genomic arsenal, and reports also suggest tests for hair are undergoing validation. The IrisPLEX assay, pioneered by a group of Dutch researchers, is legal for use in The Netherlands and takes only six genes to differentiate among 40 shades of blue or brown eye color (**Fig. 1**); in August, the group announced that they have added an assay for hair color (HIrisPLEX). Manfred Kayser, professor of forensic molecular biology at Erasmus University Medical Centre Rotterdam and leader of the VisiGen Consortium—an academic consortium dedicated to mapping the genes for human appearance—sees a future where facial features and even age can be read off DNA[1]. "That's, of course, a kind of policeman's dream, where you take a blood sample, you put it in a machine and on the computer screen you get a facial image," Kayser told a radio audience on Australia's Radio National Law Report last year.

Although commercial applications of human genomics have been focused mostly on biomedical research and have increasingly been developed for the clinic, some flagship companies are now also looking for ways to serve the law enforcement community—witness a recently announced collaboration between Illumina and the Department of Forensic and Investigative Genetics at the University of North Texas Health Science Center (Dallas)[2]—and niche companies are providing more specialized tools (**Table 1**). But the application of sequence-based testing and other high-throughput genomic assays to forensics isn't going to happen overnight. "We're not going to go from STR to sequencing in one leap," says Laurence Rubin, CEO of Identitas, a New York company with a single-nucleotide polymorphism (SNP) chips for forensic use.

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The DNA shall set you free. The individual shown, one of hundreds exonerated using DNA evidence, spent 25 years in prison after being wrongly convicted of rape. (Source: AP/Tony Gutierrez)

Citizen DNA

Of the 3 billion base pairs of information in the human genome, most are untouched by the current methods used to create DNA profiles for forensic use. Profiles stored in the US Federal Bureau of Investigation's (FBI's) Combined DNA Index System (CODIS), the United States' national storehouse of profiles created by federal, state and local crime laboratories, comprises a set of 13 short tandem repeats (STRs), 4 or 5 base pairs long, distributed across the genome (**Fig. 2**). Each STR can have several repeats (from 6 to 21) and because every person has two alleles of each STR, a profile consists of just 26 numbers representing the number of repeats at each allele. The FBI chose the individual loci based on their noncoding status, so as not to reveal personal information (personal information on subjects is held at the location where the sample was collected.) Using all 13 loci in a profile harnesses the power of statistics; the likelihood that any two individuals (except identical twins) will have the same DNA profile of all 13 loci is believed to be one in several billion (http://www.ornl.gov/sci/techresources/Human_Genome/elsi/forensics.shtml). Partial profiles of less than 13

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Figure 1 Forty shades of blue. Of 40 different blue eye colors, only the three colors in the red box couldn't be determined by IrisPlex DNA-based eye color detection system. (Reprinted with permission[3].)

loci can be useful, but do not carry the same statistical power.

As in so many things criminal, the United States now leads the world with over 10 million DNA profiles in its National DNA Index, although the United Kingdom, which was the first to create a national collection of DNA profiles, has a greater proportion of its population represented, with close to 6 million profiles (http://www.fbi.gov/about-us/lab/codis/ndis-statistics/). Under pressure from citizens' groups, the UK's Parliament passed a law last May requiring that the profiles of innocent people be removed from the database, which is expected to reduce the size of the database by more than a million profiles.

In the United States, today's collections are a combination of offender profiles and forensic profiles, or material collected at crime scenes, but in addition, several states (upward of 25) are now collecting DNA from arrestees, swelling the databases and putting stress on the local crime laboratories. That was not the intent of the US DNA Identification Act of 1994, which set up a system for collecting DNA profiles for tracking violent criminals (**Box 1** and **Fig. 3**). In addition, in some locations, DNA sweeps have been done, in which entire populations (usually of men) from prescribed areas were profiled, where law enforcement was certain of the perpetrator's location but failed to get a match in the local database. (A match can occur only if the person's DNA has been previously collected, which used to mean that he or she had already been convicted of a violent crime.) A few countries (e.g., Portugal and Denmark) have contemplated profiling their entire population; indeed, the United Arab Emirates may actually be doing it, according to GeneWatch, a UK nonprofit that monitors genetic research (**Fig. 4**).

The FBI has statistics showing that the US National DNA Index has assisted in over 200,000 criminal cases nationally. What's more, DNA profiling has been involved in exonerating over 200 prisoners, according to the Innocence Project (New York), which champions efforts to help those wrongly accused. Even so, at least two types of forensic samples yield inconclusive results with STR profiling alone: compromised DNA and mixed DNA samples. Mixtures of DNA in forensic samples occur commonly, according to the US National Institute of Standards and Technology's (NIST's) Applied Genetics Group, which assesses technologies and develops standards for forensic DNA testing. In reviewing over 5,000 DNA samples from 14 laboratories, they found that 34% of samples contained DNA from two people, and 11% contained DNA from three or four people.

Adding SNPs to the analysis enables forensic laboratories to distinguish between the genetic profiles of two individuals in a mixed sample or to make matches with compromised samples that give only partial profiles. "By simultane-

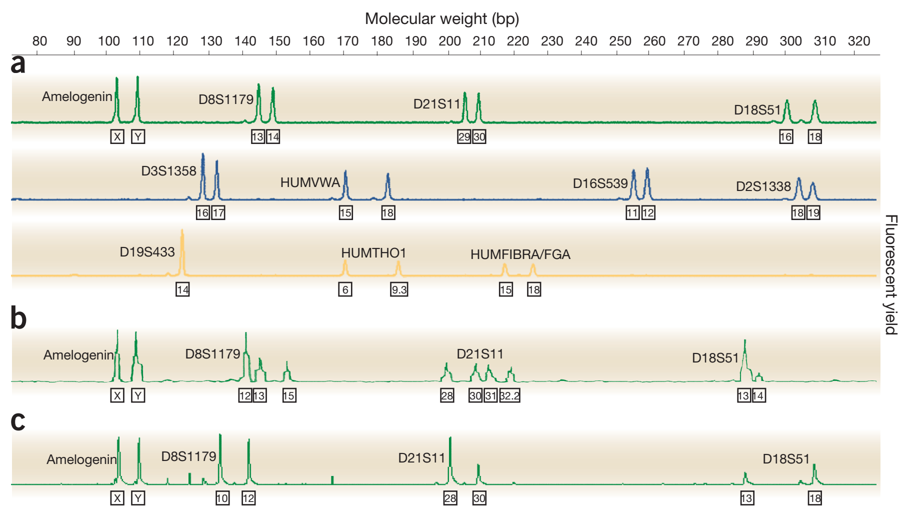

Figure 2 STR profiles. (**a**) Electropherogram of a single individual with equally balanced alleles. Numbers below the peaks indicate the number of repeats. (**b**) Mixture of two individuals in equal proportions. (**c**). Low copy number testing, where additional PCR cycles were used to overcome small sample size, leading to imbalance in alleles due to stochastic nature of PCR. (Reprinted with permission[4].)

Box 1 Mind your DNA

With DNA detection technologies becoming ever more sensitive, and DNA databases expanding their reach, the right of individuals to keep their genetic information private is being threatened. As databases increase in size, so too does the probability that an innocent person will be wrongly incriminated, a fact largely unappreciated by state legislatures and the public who believe DNA is infallible, according to William Thompson, professor in the Department of Criminology, Law and Society at the University of California, Irvine. Thompson says the possibility of error is real. DNA profiling can go off the rails in numerous ways, from contamination and mislabeling, to investigator bias, which can happen when well-intentioned people are driven by their desire to nail a person they believe is guilty[5].

Twenty-six states have enacted laws expanding their databases; among the most aggressive is California where anyone arrested for a felony must submit DNA, often under threat of further charges if they fail to provide the sample. Many of those arrested are never charged or are found innocent. Meanwhile, the California database grows by some 11,000 profiles each month. That includes people like Lily Haskell, an anti-war protester who had to give a cheek swab for a DNA test after the police arrested her for a felony. The charges were dropped, but her DNA remains in the database. With the help of the American Civil Liberties Union, she is attempting to get the law reversed[6] (*L. Haskell v. K. Harris*).

The justifications for collecting profiles simply don't hold up for people falsely accused or guilty of a lesser crime, according to Thompson. Convicted criminals forfeit some of their rights by virtue of having committed a crime, and there is a strong governmental interest in having them in a database as they are likely to commit more crimes, argues Thompson. "Neither of those rationales applies very well to people who have been arrested for some minor offense," he says.

Another potential threat to innocents is the facility with which DNA fragments can be made to order. Separately, two groups, one in Australia and the other in Israel, have produced amplicons containing CODIS fragments, using various techniques—PCR amplification from collected DNA, whole genome amplification or cloning. The Australian researchers showed that synthesized amplicons planted in a simulated crime scene, along with blood, were detectable and indistinguishable from native DNA. The Israelis, from the Tel Aviv–based company Nucleix, assembled a library of 425 CODIS fragments, sufficient to generate any profile, which also is indistinguishable from native DNA when planted at mock crime scenes. However, Nucleix has provided some solutions; their researchers showed that synthetic DNA can be distinguished from native DNA by looking for methylated bases, present only on native DNA. Whereas present-day technology for detecting methylation (bisulfite sequencing) may not be readily adaptable by forensic laboratories, they also pointed out that the presence of an unusually large number of stutters, which occur during amplification, may be another indicator of synthetic DNA, as the synthesis requires additional amplification steps.

Figure 3 Blue marks the criminal. DNA sprays can identify intruders weeks after a crime is committed. (Source: SelectaDNA, Auckland, New Zealand).

The concept of misdirecting law enforcement is not new, and there are simpler ways to do it, says Bruce Budowle. "Why go through all that, when you can just follow them around and pick up a coke can or cigarette butt?" But, argues Harvard's George Church, "those 'simpler ways' are not that much simpler, and anyway people tend to try many different ways, hoping that they can get ahead of the game. Putting anthrax spores into envelopes or ramming planes into buildings may not have seemed 'simple', but someone did it."

Turning this scenario on its head is the Kent, UK, company Selectamark Security Systems, which markets a DNA-based property marking system. Once applied to a computer or other piece of property, it cannot be completely removed, thus making it possible to identify an item as stolen and trace it back to its owner. SelectMark also offers a DNA spray for connecting intruders to a crime scene. Motion-activated devices mounted on entryways spray a solution of unique DNA on anyone entering the premises. The DNA remains visible on skin or clothing by a simple UV light for weeks (**Fig. 3**), allowing law enforcement to link a criminal to a particular crime scene. According to news reports, McDonald's fast food restaurants in Australia and the Netherlands are testing the system. *LD*

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ously interrogating SNPs selected for identification, more information can be obtained from partially degraded samples that are currently deemed 'inconclusive' and thus a dead end for the justice system," says Cydne Holt, senior market manager for applied markets at Illumina and former director of San Francisco's crime laboratory.

Using current technologies to reanalyze old DNA samples that previously had given inconclusive results could have life-altering consequences for the wrongly convicted. In a recent case in Fort Worth, Texas, David Wiggins, a prisoner held since being sentenced to life in 1989 for aggravated assault of a 14-year-old girl, was exonerated when new technologies for isolating sperm cells and interrogating Y-chromosome STRs was applied to a semen stain on the victim's clothing. The Innocence Project took on Wiggins' case in 2007, but it wasn't until this year that they finally got the evidence they needed to exonerate him. "Advances in DNA technology have come into play in a lot of our cases," says Paul Cates, communications director at the Innocence Project. "It's not unusual for us to have cases where the technology has improved over the years and ultimately helps someone."

Wiggins was fortunate that the evolution of DNA analysis technology was on his side. For many others, DNA samples that might exonerate them are still intractable with today's technology. "There are some cases that we have to close because of inconclusive results, for example, because the sample was too old, degraded or there was not enough DNA to test," says Cates. So whereas advances in DNA technology have been helpful, there's room for improvement. "Nearly one in five of our cases are dropped because of difficulty in analyzing the DNA, so from our perspective, there is room for techniques that could better analyze difficult samples," says Cates.

Another modification to CODIS that has been useful, particularly with degraded samples, are mini-STRs. Researchers at NIST developed a set of mini-STRs for all 13 CODIS loci that require samples be only 100 base pairs long, by designing PCR primers that bind closer to the repeat. (Standard STR analysis requires 400 base pair fragments.) These were

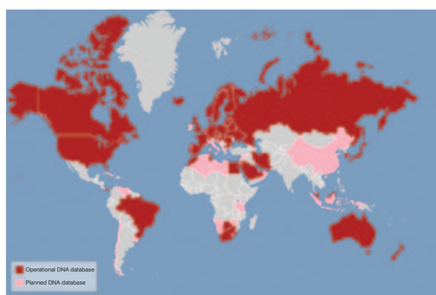

Figure 4 Locations of national DNA databases. Dark shading: pperational DNA database; light shading: planned DNA database. (Source: Council for Responsible Genetics, Cambridge, MA, USA)

particularly helpful during the effort to identify victims of the World Trade Center disaster. Only 655 people of the estimated 2,753 victims could be identified using standard DNA profiling techniques due to the intense heat at the site and contamination with inorganic building material, which left many samples too degraded to analyze by standard methods. Forensic scientists in the New York City Office of Chief Medical Examiner turned to other tools, including SNP analysis, mini-STRs and mitochondrial DNA, bringing the number of 9/11 victims identified by DNA analysis to 1,633 people. Further improvements to isolation methods and analytical tools were contributed by a number of private companies, among them Cybergenetics (Pittsburgh), Orchid Cellmark (Princeton, NJ, USA), a division of Orchid Biosciences, Myriad Genetics (Salt Lake City, UT), Celera Genomics (Alameda, CA, USA) and Bode Technology (Lorton, VA, USA).

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Unblocking the backlog

Greater demand, coupled with more evidence being collected by law enforcement, has created a backlog of DNA cases. According to the National Institute of Justice (NIJ), a case becomes backlogged when the sample has not been analyzed 30 days after submission to the laboratory. With increases in throughput available with next generation sequencing (NGS) platforms, backlogs could be reduced or eliminated. Illumina's MiSeq sequencer, which uses as little as 50 ng DNA as input, can analyze all the loci used in forensic laboratories worldwide, plus hundreds more—in a single run. "This includes the core sets of autosomal and Y STRs (as dictated by each nation), many additional STRs, including those on the X chromosome, several categories of SNPs and the mitochondrial DNA genome, as well as other classes of polymorphisms," says Holt. Likewise Life Technologies' (Carlsbad, CA, USA) Ion PGM sequencer, which can use as little as 10 ng input DNA along with the multiplexing capabilities of its Ion AmpliSeq Target Selection Technology, lets you use as many as 1,536 primers in a single tube. "The fact that the library prep method for PGM requires at least 15 times less DNA than other NGS methods is a big advantage," claims John Gerace, head of applied sciences for Life Technologies.

Michael Sheppo, director of the Office of Investigative and Forensic Sciences at the NIJ, acknowledges the benefits that NGS could bring to forensics but recognizes that challenges remain. "The potential advantage for performing highly multiplexed sequencing reactions that could produce information from several marker systems simultaneously presents a strong argument for replacing current methods with NGS systems." But, two major concerns with the technology are data quality and the length of the reads. "The quality of the sequence has direct relevance to the confidence that the data generated can be used in court, and the length of the read has direct relationship to what kinds of markers can be analyzed with the method," he says.

Monte Miller, president of the consulting firm Forensic DNA Experts (Riverside, CA, USA), feels that speed, cost and precision are at issue. "If you could sequence the 13 loci more quickly and efficiently, and you got the same power of statistics, that's more likely to happen first—so that they don't have to change CODIS right off the bat," he says. He also notes that for each allele used by CODIS, the frequencies in the general population, and various subpopulations, are known. This is required to estimate the likelihood that a DNA sample came from a particular person. These statistics would have to be gathered anew if the profiling system were changed drastically.

As technology for DNA detection devices matures, law enforcement may someday be able to process crime scenes on the spot (**Box 2**). But what forensics really needs is a technology that is not dependent on PCR, according to Rotterdam's Kayser. "The real breakthrough will come when PCR can be avoided in NGS—all current studies use PCR-based NGS—as slippage artifacts occurring during PCR can cause problems [because] it cannot necessarily be known whether a small PCR [capillary electrophoresis] peak comes from a real allele (that is, an additional contributor) or from slippage artifacts," he says.

CODIS and beyond

There's no disputing the benefit that DNA profiling has provided law enforcement. "CODIS has been a fantastic tool for law enforcement for many years," says David Whelan, an investor and director at Identitas. "However, when you run a sample and you get no match against a known reference sample, that's the end of the line," he says. And that's where some biotechs are placing their bets, with developing technologies to bridge this gap.

Identitas has developed a high-density array based on Illumina's genotyping chip technology that provides information for no-match samples. "We can say they are of a certain

Table 1 Companies developing technologies with forensic applications

Company (location)	Product
Illumina	SNP genotyping sequencing services
Life Technologies	AutoMate Express benchtop DNA extraction system for forensics with AmpliFSTR Identifiler Plus PCR amplification kit
Promega (Madison, WI, USA)	STR systems to amplify CODIS loci, kits for sample preparation and DNA quantification
Qiagen (Hilden, Germany)	QuantiPlex Hyres kit, sample extraction solutions optimized for forensic samples
AI Genetics (Fairfax Identity Laboratories) (Richmond, VA, USA)	Full-service forensic laboratory in addition to other offerings in DTC genomic testing, relationship, and CLIA clinical genetics laboratory
Casework Genetics (Woodbridge, VA, USA)	Ultra high-density SNP arrays using Illumina Human Omni1-Quad Beadchip
Cybergenetics (Pittsburgh)	TrueAllele software package for casework technology, TrueAllele Databank
DNA Diagnostics Center (Fairfield, OH, USA)	DNA testing for forensic, paternity, ancestry, immigration, accredited by the American Society of Crime Laboratory Directors
Gene Codes Forensics (Ann Arbor, MI, USA)	Sequencer software package adapted for forensics work with mitochondrial DNA analysis
Identitas	Developing high-density SNP chip for forensic market
ZyGem (Hamilton, New Zealand and Charlottesville, VA, USA)	Markets *forensicsGEM* high-throughput DNA extraction kit which is compatible with STR profiling kits

DTC, direct to consumer; CLIA, Clinical Laboratory Improvement Amendments.

Box 2 DNA profiles on demand

Many sequencing companies are racing to be the first to market with a portable, turnkey-type sequencer that can generate DNA profiles at the crime scene. This requires that the system be easy to use, and hardy enough to be transported and used by law enforcement personnel who likely lack scientific training. Instruments that fit the bill are just emerging. For example, IntegenX (Pleasanton, CA, USA) recently released their RapidHIT instrument, which conducts STR-based profiling in fewer than 90 min without a highly trained operator.

Partnering with Key Forensic Services in the United Kingdom for the initial implementation, IntegenX hopes to make RapidHIT accessible to law enforcement personnel. "Key Forensic Services are a perfect partner to both initially use and help implement law enforcement custody suite usage of rapid DNA identity systems, and in future help extend the usage to crime scene stains," says Stevan Jovanovich, president and CEO of IntegenX. Other companies developing rapid DNA sequencing systems for a variety of applications include Lockheed Martin (Bethesda, MD, USA) and ZyGEM (Hamilton, New Zealand), which together are developing a rapid DNA analysis cartridge, QuantuMDx (Tyne and Wear, UK), which has a nanowire-based point-of-care instrument, Q-POC and DNA Electronics (London) and geneOnyx (London), which are combing forces to create a device for on-site analysis for cosmetic purposes. Sandia National Laboratories (Albuquerque, NM, USA), a US government research facility, offer the Battlefield Automated DNA Analysis and Sampling System, a customized, droplet-based, digital microfluidic platform that can be used by soldiers with little scientific experience to analyze DNA samples on the battlefield.

The fierce competition to be first to, and best in, the market for rapid DNA sequencers can only further the overall goal of improving law enforcement. "The main challenge facing law enforcement is timely information," says Jovanovich. "PCR is a technology that enables the analysis of vanishingly small amounts of DNA, but the law enforcement investigator needs information as soon as possible so that the crime scene does not get cold. IntegenX has integrated eight steps, including PCR, to streamline the determination of identity information to help catch bad guys faster." In October, the company released the instrument for sale in the United States. *CS*

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ethnic background…are related to somebody else that [we] have another sample for—which is very important—as well as [identify] external, phenotypic traits that can really help [law enforcement] focus," says Whelan. Results of a pilot study of over 3,000 profiles, done in collaboration with the VisiGen Consortium as well as several law enforcement agencies, which provided the samples, will be released shortly. The study looks at gender, first- and third-degree relatedness and geographic ancestry, and builds up a visual profile of the subject. "The agencies that contributed the data were very impressed with the results," says Identitas CEO Rubin.

Others are working to improve the ability to deconvolute mixtures, which can also lead to dead ends. Cybergenetics (Pittsburgh) has developed a software package, TrueAllele, which can take previously unanalyzable samples and give results that can be used with existing law enforcement tools. TrueAllele automates the analysis of raw STR data; using Markov chain modeling, it takes features such as peak height, shape and area, and calculates the probabilities that particular genotypes comprise complex profiles.

Not so fast

Another potential roadblock for incorporation of NGS into forensics involves privacy issues, especially where governments are involved. "Would the generation of additional data from genetic markers that might be linked to medical information result in privacy concerns?" asks Sheppo. Peter de Knijff, professor at the Forensic Laboratory for DNA Research at Leiden University Medical Centre in The Netherlands, whose laboratory is actively involved in advising the Ministry of Safety and Justice in The Netherlands about possible future uses of NGS-based methods, says, "Legislation and ethics issues relating to the unlimited genetic information one could infer from NGS DNA profiles will be a major barrier in many countries."

Table 2 Commercial DNA testing laboratories

Forensics-focused companies[a] (location)	Identity/relationship focused companies[b] (location)
Andergene Labs (Oceanside, CA, USA)	Affiliated Genetics
Anjura Technology (STACSDNA) (Fairfax, VA, USA	BRT Laboratories (Baltimore)
Bode Technology Group	Cellmark DNA Paternity Services (Oxfordshire, UK)
Cybergenetics	DNA Findings (Houston)
DNA Clinics (London)	DNA Heritage (Houston)
DNA Diagnostics Center (Fairfield, OH, USA)	DNA Services of America (multiple sites in the US)
DNA Reference Laboratory (San Antonio, TX, USA)	easyDNA (Elk Grove, CA, USA)
DNA Resource (Washington, DC, USA)	Family Tree DNA (Houston)
DNA Security (Burlington, NC, USA)	Genetrack Biolabs (Vancouver, BC, Canada)
DNA Solutions (multiple global sites)	Genetic Profiles (San Diego)
DNA Testing Solutions (Tampa, FL, USA)	Genetic Testing Laboratories (Las Cruces, NM, USA)
DNA Worldwide (Frome, UK)	GeneTree DNA Testing Center (Salt Lake City, UT, USA)
Fairfax Identity Labs (Richmond, VA, USA)	Identigene (Salt Lake City, UT, USA)
Forensic Bioinformatics (Fairborn, OH, USA)	Identity Genetics (Aurora, SD, USA)
Forensic DNA Experts	LabsDirect (multiple sites in UK)
Forensic Science Associates (Richmond, CA, USA)	Long Beach Genetics Esoterix (Rancho Dominguez, CA, USA)
Future Technologies (Fairfax, VA, USA)	Oxford Ancestors (Oxford, UK)
Gene Codes Forensics	Paternity Testing Corporation (Columbia, MO, USA)
Genetic Technologies (Glenco, MO, USA)	Sorenson Genomics (Salt Lake City, UT, USA)
Mitotyping Technologies (State College, PA, USA)	
Molecular World (Laval, Quebec)	
Myriad Genetic Laboratories	
Orchid Cellmark (multiple global sites)	
QuestGen Forensics (Davis, CA, USA)	
PRO-DNA Diagnostic (Laval, Quebec)	
SoftGenetics (State College, PA, USA)	
Sozer, Niezoda and Associates (Alexandria, VA, USA)	

[a]Provides services to forensic laboratories. [b]Provides services to individuals seeking information on ancestry and paternity.

Box 3 Unlocking mysterious deaths

In the popular mind, forensic science is associated with the examination of a crime scene and presentation of evidence found there at a trial. But many of forensic scientists' investigations are related to answering a much more basic question. What do you fill in on a death certificate after the words "cause of death"?

In over a little more than a decade, dramatic advances in both the technology of genetic testing and in our understanding of the genetics of certain conditions have given rise to a new way for coroners, medical examiners and pathologists to explain what have traditionally been the most troubling of deaths—so-called autopsy-negative sudden unexplained deaths (SUDs). SUDS are the incidences where seemingly healthy and symptomless people, largely between the ages of 1 and 35, keel over and die. When traditional physical, toxicological, metabolic screens are done, no physical obvious cause of death can be found. Conducting what have come to be called 'molecular autopsies'—largely tests for genes related to heart disease carried by the dead person—forensic scientists have been able to associate many previously mysterious deaths with heart arrhythmias that are known to strike and kill without any previous warning. But equally important, because there often are ways to prevent the heart attacks, molecular autopsies are being used as a pretext to test close relatives of the dead person for the deadly mutation and physical manifestations of the disease.

A mark of the speed of molecular autopsies application is that in 1999 Mayo Clinic pediatric cardiologist Michael Ackerman and his colleagues in Rochester, Minnesota, reported conducting the world's first such autopsy on a 19-year-old woman and then linking her death to a gene mutation that her sister also carried. They predicted that their discovery "holds potentially great importance for forensic science"[7].

The blooming of that potential appeared in June when Ackerman and his colleagues reported that they had looked at samples of SUD cases sent to them over a 12-year period by medical examiners. When a molecular autopsy was conducted, mutations previously identified as pathogenic were identified in 26% of cases[8].

Equally significant from a biotech perspective, genetic testing companies and laboratories—GeneDx, Partners Healthcare Center, Transgenomics and others—have over the past five to seven years begun to offer post-mortem tests both to detect deleterious mutations and to promote this testing. "We regularly go to medical examiners' meetings," remarks Sherri Bale, the managing director of GeneDx in Gaithersburg, Maryland.

Impressed with the promise of the analysis, some medical pathology offices are moving to make a molecular autopsy something like standard operating procedure. The Ontario Forensic Pathology Service has put in place a facility in Toronto to systematically collect, analyze and store tissues taken from SUD victims whose cause of death could not otherwise be determined.

And yet with all of these advances, when you talk to people in the field, whether researchers, genetic testing companies, coroners or medical examiners, there is a sense that the genetic autopsy revolution hoped for since 1999 is still idling in neutral gear. Part of this has to do with the necessity of a fundamental reconfiguration in how medical examiners and coroners conceive of their work and conduct their autopsies.

One issue is preservation of material. "One of the challenges is that the vast majority of tissue samples from autopsies are complete failures in genetic testing," says Heidi Rehm, director of the Harvard-affiliated Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine in Cambridge Massachusetts. The formalin traditionally used to preserve body tissues in autopsies destroys the genetic reliability of the sample.

Equally importantly, a forensic autopsy traditionally is used to rule out a criminal cause of death whereas a genetic explanation for a SUD carries with it an implicit 'duty to warn' responsibility to living family members. This challenges medical examiners and coroners, many of whom have no medical training and in North America are often political appointees, to change into something they have never been before—physicians.

Silvia Priori, professor of medicine at the New York University School of Medicine, has worked closely with the New York City Office of Chief Medical Examiner, which has developed an expertise in genetic testing, as a result of their efforts to identify remains after the 9/11 attacks. She says follow-up testing on family members whom an autopsy indicates could be carrying a lethal mutation is not taking place because medical examiners "aren't organized to do a follow-up."

Not all coroners are equally stuck. In Ontario, provincial forensic pathologist Kris Cunningham says his department has put in place a protocol where close family members will be told if a mutation has been found in a deceased relative that they may carry. And they will also be counseled to go to a doctor for an examination and possibly a genetic test.

Circling about all this comes the issue of who pays for a molecular autopsy which, depending on the test, can cost anywhere between $2,500 and $9,000. In most places around the world, both private medical insurance and government-covered health coverage ceases at death. This means that families wanting to learn if there is genetic explanation for the unexpected death of a loved one usually have to pay themselves.

In response to economic issues coroners and grieving families regularly try to convince research institutions to slip molecular autopsies onto their research budgets even though the testing, "is not really a research question any longer...it is a clinical question," says Ackerman.

And none of this addresses the most confusing question of all. Whereas in some diseases, the link between a mutation and a potentially fatal condition has been strongly made—mutations in three genes explain roughly 75% of all Long QT cases (a rare potentially serious heart condition spotted by irregular EKGs)—in many conditions the linkage between genetics and the course of a genetically inherited disease is extremely amorphous. For example, mutations that can cause death in some arrhythmogenic right ventricular cardiomyopathy carriers are apparently benign in others. But even more troubling are the hundreds and hundreds of "mutations of unknown clinical significance" which regularly are found when general screenings of genes linked to the rare heart conditions are made.

All this has led cardiologists to walk softly when it comes to routinizing molecular autopsies. "In the setting of autopsy-negative SUDS...testing may be considered in an attempt to establish probable cause and manner of death and to facilitate the identification of potentially at-risk relatives," is how a consensus paper by European and North American cardiologists put it last year[9].

So what is the way forward? One answer may be showing that however expensive it is, molecular autopsies are still cheaper than a continual physical testing of living family members who genetically may be at risk for SUDs. It is an analysis that Ackerman is working on based on the 173 cases and he says the results will hopefully be published soon. *SS*

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Others downplay concerns about storing private information that might be used against people. "There is, of course, the worry about genetic information being used by others to stigmatize and discriminate, but that means someone would have to get access to a person's genomic data and so far I don't see that being very easy to do. People already are stigmatized and discriminated against without anyone knowing their genetic information," says Karen Maschke, research scholar at the Hastings Center (Garrison, NY, USA).

Fight on

Identity testing has become a cottage industry, with a host of companies offering genetic testing for various legal reasons—paternity, immigration—whereas others cater to the needs of law enforcement (**Table 2**). "If popular culture and media are the meters by which we measure society's feeling toward a science, it is clear that society is very interested in the forensic sciences," says NIJ's Sheppo. And the benefits go beyond criminal applications. DNA technology has been brought to bear in other areas of forensic sciences, such as solving cases of unexplained deaths (**Box 3**).

But as with most areas of science, levels of funding are linked to technology advancement. It has taken government support in the past to advance major improvements in the forensic sciences. In 2006, the NIJ provided over $107 million to fund a five-year study, which supported the expansion of forensic DNA applications in state and local laboratories, bringing capillary electrophoresis and robotic automation, as well as many additional technological advances to state and local forensic laboratories. NIJ's Sheppo points out, "Without this kind of government support, it is difficult to imagine that forensic DNA laboratories would have been able to expand in the way that they have over the last decade." But there are still areas in need of improvement. According to Bruce Budowle, director of the University of North Texas Health Science Center's Institute of Investigative Genetics, "The limitation with CODIS is [that it is] driving casework rather than casework driving CODIS."

It's not clear where the next set of advances will come. "The early adopters may not be in law enforcement," says Kevin Lothridge, CEO of the National Forensic Science Technology Center (Largo, FL, USA), a nonprofit agency that provides training and technology assessment. "They may be in other arenas that use forensics and biometrics, such as Homeland Security, the Department of Defense or [the] US border patrol."

Harvard's George Church finds that the potential benefits justify the efforts. "The issue is not whether the new forensic technology is perfect, but whether it is better than eyewitness sketches, etc. The same is true for new diagnostics—the issue is not how many people get no medical insight, but rather the number of patients who are helped by the new technology," says Church.

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