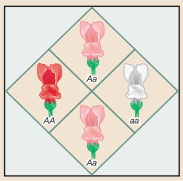
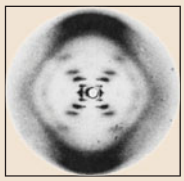
PART 1

HISTORY





OUTLINE

UNLIKE THE REST OF THIS BOOK, the two chapters that make up Part 1 contain material largely unchanged from earlier editions. We nevertheless keep these chapters because the material remains as important as ever. Specifically, Chapters 1 and 2 provide an historical account of how the field of genetics and the molecular basis of genetics were established. Key ideas and experiments are described.

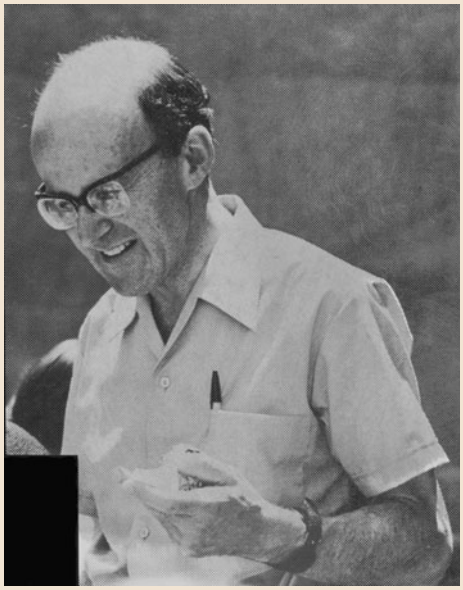
Chapter 1 addresses the founding events in the history of genetics. We discuss everything from Mendel's famous experiments on peas, which uncovered the basic laws of heredity, to the one gene encodes one enzyme hypothesis of Garrod. Chapter 2 describes the revolutionary development of molecular biology that was started with Avery's discovery that DNA was the genetic material, and continued with James Watson and Francis Crick's proposal that the structure of DNA is a double helix, and the elucidation of the genetic code and the "central dogma" (DNA "makes" RNA which "makes" protein). Chapter 2 concludes with a discussion of recent developments stemming from the complete sequencing of the genomes of many organisms and the impact this sequencing has on modern biology.

PHOTOS FROM THE COLD SPRING HARBOR LABORATORY ARCHIVES

Vernon Ingram, Marshall W. Nirenberg, and Matthias Staehelin, 1963 Symposium on Synthesis and Structure of Macromolecules. Ingram demonstrated that genes control the amino acid sequence of proteins; the mutation causing sickle-cell anemia produces a single amino acid change in the hemoglobin protein (Chapter 2). Nirenberg was key in unraveling the genetic code, using protein synthesis directed by artificial RNA templates in vitro (Chapters 2 and 16). For this achievement, he shared in the 1968 Nobel Prize in Physiology or Medicine. Staehelin worked on the small RNA molecules, tRNAs, which translate the genetic code into amino acid sequences of proteins (Chapters 2 and 16).

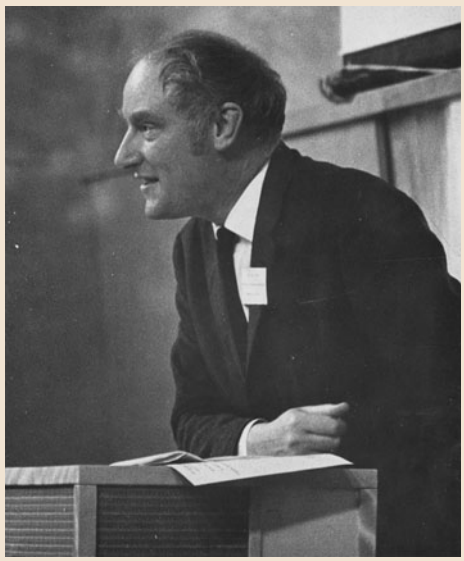
Melvin Calvin, Francis Crick, George Gamow, and James Watson, 1963 Symposium on Synthesis and Structure of Macromolecules. Calvin won the 1961 Nobel Prize in Chemistry for his work on CO_2 assimilation by plants. For their proposed structure of DNA, Crick and Watson shared in the 1962 Nobel Prize in Physiology or Medicine (Chapters 2 and 4). Gamow, a physicist attracted to the problem of the genetic code (Chapters 2 and 16), founded an informal group of like-minded scientists called the RNA Tie Club. (He is wearing the club tie, which he designed, in this picture.)

Raymond Appleyard, George Bowen, and Martha Chase, 1953 Symposium on Viruses. Appleyard and Bowen, both phage geneticists, are here shown with Chase, who, in 1952, together with Alfred Hershey, did the simple experiment that finally convinced most people that the genetic material is DNA (Chapter 2).



Max Perutz, 1971 Symposium on Structure and Function of Proteins at the Three-Dimensional Level. Perutz shared, with John Kendrew, the 1962 Nobel Prize for Chemistry; using X-ray crystallography, and after 25 years of effort, they were the first to solve the atomic structures of proteins—hemoglobin and myoglobin, respectively (Chapter 6).

Sydney Brenner and James Watson, 1975 Symposium on The Synapse. Brenner, shown here with Watson, contributed to the discoveries of mRNA and the nature of the genetic code (Chapters 2 and 16); his share of a Nobel Prize, in 2002, however, was for establishing the worm, *Caenorhabditis elegans*, as a model system for the study of developmental biology (Appendix 1).



Francis Crick, 1963 Symposium on Synthesis and Structure of Macromolecules. In addition to his role in solving the structure of DNA, Crick was an intellectual driving force in the development of molecular biology during the field's critical early years. His "adaptor hypothesis" (published in the RNA Tie Club newsletter) predicted the existence of molecules required to translate the genetic code of RNA into the amino acid sequence of proteins. Only later were tRNAs found to do just that (Chapter 15).

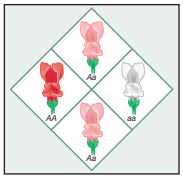
Seymour Benzer, 1975 Symposium on The Synapse. Using phage genetics, Benzer defined the smallest unit of mutation, which turned out later to be a single nucleotide (Chapter 1 and Appendix 1). This same work also provided an experimental definition of the gene—which he called a cistron—using functional complementation tests. Later, his studies focused on behavior, using the fruit fly as a model.

Calvin Bridges, 1934 Symposium on Aspects of Growth. Bridges (shown reading the newspaper) was part of T.H. Morgan's famous "fly group" that pioneered the development of the fruit fly *Drosophila* as a model genetic organism (Chapter 1 and Appendix 1). With him is John T. Buchholtz, a plant geneticist who was a summer visitor at CSHL at the time, and who, in 1941, became President of the Botanical Society of America.

Charles Yanofsky, 1966 Symposium on The Genetic Code. Yanofsky (right), together with Sydney Brenner, proved colinearity of the gene—that is, that successive groups of nucleotides encoded successive amino acids in the protein product (Chapter 2). He later discovered the first example of transcriptional regulation by RNA structure in his detailed analysis of attenuation at the tryptophan operon of *Escherichia coli* (Chapter 20). He is pictured here talking to Michael Chamberlin, who studied transcription initiation by RNA polymerase.

Edwin Chargaff, 1947 Symposium on Nucleic Acids and Nucleoproteins. The eminent nucleic acid biochemist Chargaff's famous ratios—that the amount of adenine in a DNA sample matched that of thymine, and the amount of cytosine matched that of guanine—were later understood in the context of Watson and Crick's DNA double helix structure. Perhaps frustrated that he had never come up with base pairs himself, he became a bitter critic of molecular biology, an occupation he described as "essentially the practice of biochemistry without a license."

CHAPTER 1



The Mendelian View of the World

IT IS EASY TO CONSIDER HUMAN BEINGS UNIQUE among living organisms. We alone have developed complicated languages that allow meaningful and complex interplay of ideas and emotions. Great civilizations have developed and changed our world's environment in ways inconceivable for any other form of life. There has always been a tendency, therefore, to think that something special differentiates humans from every other species. This belief has found expression in the many forms of religion through which we seek the origin of and explore the reasons for our existence and, in so doing, try to create workable rules for conducting our lives. Little more than a century ago, it seemed natural to think that, just as every human life begins and ends at a fixed time, the human species and all other forms of life must also have been created at a fixed moment.

This belief was first seriously questioned almost 150 years ago, when Charles Darwin and Alfred R. Wallace proposed their theories of evolution, based on the selection of the most fit. They stated that the various forms of life are not constant but continually give rise to slightly different animals and plants, some of which adapt to survive and multiply more effectively. At the time of this theory, they did not know the origin of this continuous variation, but they did correctly realize that these new characteristics must persist in the progeny if such variations are to form the basis of evolution.

At first, there was a great furor against Darwin, most of it coming from people who did not like to believe that humans and the rather obscene-looking apes could have a common ancestor, even if this ancestor had lived some 10 million years ago. There was also initial opposition from many biologists who failed to find Darwin's evidence convincing. Among these was the famous naturalist Jean L. Agassiz, then at Harvard, who spent many years writing against Darwin and Darwin's champion, Thomas H. Huxley, the most successful of the popularizers of evolution. But by the end of the 19th century, the scientific argument was almost complete; both the current geographic distribution of plants and animals and their selective occurrence in the fossil records of the geologic past were explicable only by postulating that continuously evolving groups of organisms had descended from a common ancestor. Today, evolution is an accepted fact for everyone except a fundamentalist minority, whose objections are based not on reasoning but on doctrinaire adherence to religious principles.

An immediate consequence of Darwinian theory is the realization that life first existed on our Earth more than 4 billion years ago in a simple

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form, possibly resembling the bacteria—the simplest variety of life known today. The existence of such small bacteria tells us that the essence of the living state is found in very small organisms. Evolutionary theory further suggests that the basic principles of life apply to all living forms.

MENDEL'S DISCOVERIES

Gregor Mendel's experiments traced the results of breeding experiments (genetic crosses) between strains of peas differing in well-defined characteristics, like seed shape (round or wrinkled), seed color (yellow or green), pod shape (inflated or wrinkled), and stem length (long or short). His concentration on well-defined differences was of great importance; many breeders had previously tried to follow the inheritance of more gross qualities, like body weight, and were unable to discover any simple rules about their transmission from parents to offspring (see Box 1-1, Mendelian Laws).

The Principle of Independent Segregation

After ascertaining that each type of parental strain bred true—that is, produced progeny with particular qualities identical to those of the parents—Mendel performed a number of crosses between parents (P) differing in single characteristics (such as seed shape or seed color). All the progeny (F_1 = first filial generation) had the appearance of *one* parent only. For example, in a cross between peas having yellow seeds and peas having green seeds, all the progeny had yellow seeds. The trait that appears in the F_1 progeny is called **dominant**, whereas the trait that does not appear in F_1 is called **recessive**.

ADVANCED CONCEPTS

Box 1-1 Mendelian Laws

The most striking attribute of a living cell is its ability to transmit hereditary properties from one cell generation to another. The existence of heredity must have been noticed by early humans, who witnessed the passing of characteristics, like eye or hair color, from parents to offspring. Its physical basis, however, was not understood until the first years of the 20th century, when, during a remarkable period of creative activity, the chromosomal theory of heredity was established.

Hereditary transmission through the sperm and egg became known by 1860, and in 1868 Ernst Haeckel, noting that sperm consists largely of nuclear material, postulated that the nucleus is responsible for heredity. Almost 20 years passed before the chromosomes were singled out as the active factors, because the details of mitosis, meiosis, and fertilization had to be worked out first. When this was accomplished, it could be seen that, unlike other cellular constituents, the chromosomes are equally divided between daughter cells. Moreover, the complicated chromosomal changes that reduce the sperm and egg chromosome number to the haploid number during meiosis became understandable as necessary for keeping the chromosome number constant. These facts, however, merely suggested that chromosomes carry heredity.

Proof came at the turn of the century with the discovery of the basic rules of heredity. The concepts were first proposed by Gregor Mendel in 1865 in a paper entitled "Experiments in Plant Hybridization" given to the Natural Science Society at Brno. In his presentation, Mendel described in great detail the patterns of transmission of traits in pea plants, his conclusions of the principles of heredity, and their relevance to the controversial theories of evolution. The climate of scientific opinion, however, was not favorable, and these ideas were completely ignored, despite some early efforts on Mendel's part to interest the prominent biologists of his time. In 1900, 16 years after Mendel's death, three plant breeders working independently on different systems confirmed the significance of Mendel's forgotten work. Hugo de Vries, Karl Correns, and Erich von Tschermak-Seysenegg, all doing experiments related to Mendel's, reached similar conclusions before they knew of Mendel's work.

The meaning of these results became clear when Mendel set up genetic crosses between F_1 offspring. These crosses gave the important result that the recessive trait reappeared in approximately 25% of the F_2 progeny, whereas the dominant trait appeared in 75% of these offspring. For each of the seven traits he followed, the ratio in F_2 of dominant to recessive traits was always approximately 3:1. When these experiments were carried to a third (F_3) progeny generation, all the F_2 peas with recessive traits bred true (produced progeny with the recessive traits). Those with dominant traits fell into two groups: one third bred true (produced only progeny with the dominant trait); the remaining two-thirds again produced mixed progeny in a 3:1 ratio of dominant to recessive.

Mendel correctly interpreted his results as follows (Fig. 1-1): the various traits are controlled by pairs of factors (which we now call **genes**), one factor derived from the male parent, the other from the female. For example, pure-breeding strains of round peas contain two versions (or **alleles**) of the roundness gene (*RR*), whereas pure-breeding wrinkled strains have two copies of the wrinkledness (*rr*) allele. The round-strain gametes each have one gene for roundness (*R*); the wrinkled-strain gametes each have one gene for wrinkledness (*r*). In a cross between *RR* and *rr*, fertilization produces an F_1 plant with both alleles (*Rr*). The seeds look round because *R* is dominant over *r*. We refer to the appearance or physical structure of an individual as its **phenotype**, and to its genetic composition as its **genotype**. Individuals with identical phenotypes may possess different genotypes; thus, to determine the genotype of an organism, it is frequently necessary to perform genetic crosses for several generations. The term **homozygous** refers to a gene pair in which both the maternal and paternal genes are identical (e.g., *RR* or *rr*). In contrast, those gene pairs in which paternal and maternal genes are different (e.g., *Rr*) are called **heterozygous**.

One or several letters or symbols may be used to represent a particular gene. The dominant allele of the gene may be indicated by a capital letter (*R*), by a superscript + (r^+), or by a + standing alone. In our discussions here, we use the first convention in which the dominant allele is represented by a capital letter and the recessive allele by the lowercase letter.

It is important to notice that a given gamete contains only one of the two copies (one allele) of the genes present in the organism it comes from (e.g., either *R* or *r*, but never both) and that the two types of gametes are produced in equal numbers. Thus, there is a 50:50 chance that a given gamete from an F_1 pea will contain a particular gene (*R* or *r*). This choice is purely random. We do not expect to find *exact* 3:1 ratios when we examine a limited number of F_2 progeny. The ratio will sometimes be slightly higher and other times slightly lower. But as we look at increasingly larger samples, we expect that the ratio of peas with the dominant trait to peas with the recessive trait will approximate the 3:1 ratio more and more closely.

The reappearance of the recessive characteristic in the F_2 generation indicates that recessive alleles are neither modified nor lost in the F_1 (*Rr*) generation, but that the dominant and recessive genes are independently transmitted and so are able to segregate independently during the formation of sex cells. This **principle of independent segregation** is frequently referred to as Mendel's first law.

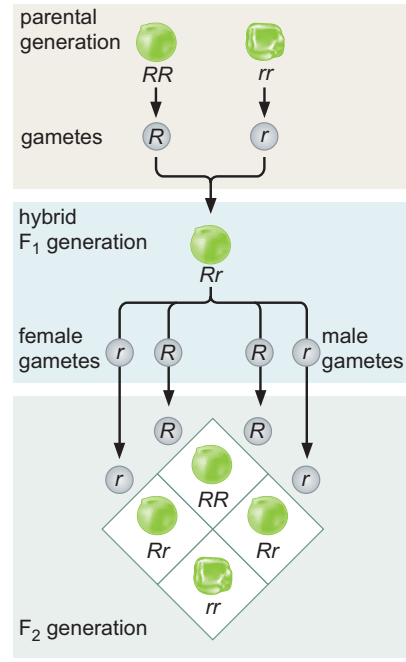


FIGURE 1-1 How Mendel's first law (independent segregation) explains the 3:1 ratio of dominant to recessive phenotypes among the F_2 progeny. *R* represents the dominant gene and *r* the recessive gene. The round seed represents the dominant phenotype, the wrinkled seed the recessive phenotype.

Some Alleles Are neither Dominant nor Recessive

In the crosses reported by Mendel, one member of each gene pair was clearly dominant to the other. Such behavior, however, is not universal. Sometimes the heterozygous phenotype is intermediate between the two homozygous

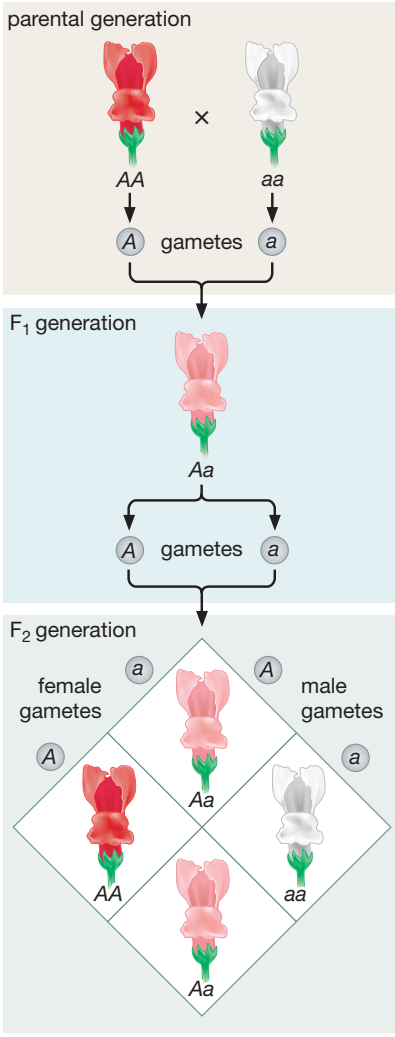


FIGURE 1-2 The inheritance of flower color in the snapdragon. One parent is homozygous for red flowers (*AA*) and the other homozygous for white flowers (*aa*). No dominance is present, and the heterozygous F_1 flowers are pink. The 1:2:1 ratio of red, pink, and white flowers in the F_2 progeny is shown by appropriate coloring.

phenotypes. For example, the cross between a pure-breeding red snapdragon (*Antirrhinum*) and a pure-breeding white variety gives F_1 progeny of the intermediate pink color. If these F_1 progeny are crossed among themselves, the resulting F_2 progeny contain red, pink, and white flowers in the proportion of 1:2:1 (Fig. 1-2). Thus, it is possible here to distinguish heterozygotes from homozygotes by their phenotype. We also see that Mendel's laws do not depend on whether one allele of a gene pair is dominant over the other.

Principle of Independent Assortment

Mendel extended his breeding experiments to peas differing by more than one characteristic. As before, he started with two strains of peas, each of which bred pure when mated with itself. One of the strains had round yellow seeds; the other, wrinkled green seeds. Since round and yellow are dominant over wrinkled and green, the entire F_1 generation produced round yellow seeds. The F_1 generation was then crossed within itself to produce a number of F_2 progeny, which were examined for seed appearance (phenotype). In addition to the two original phenotypes (round yellow; wrinkled green), two new types (**recombinants**) emerged: wrinkled yellow and round green.

Again Mendel found he could interpret the results by the postulate of genes, if he assumed that each gene pair was independently transmitted to the gamete during sex-cell formation. This interpretation is shown in Figure 1-3. Any one gamete contains only one type of allele from each gene pair. Thus, the gametes produced by an F_1 (*RrYy*) will have the composition *RY, Ry, rY*, or *ry*, but never *Rr, Yy, YY*, or *RR*. Furthermore, in this example, all four possible gametes are produced with equal frequency. There is no tendency of genes arising from one parent to stay together. As a result, the F_2 progeny phenotypes appear in the ratio nine round yellow, three round green, three wrinkled yellow, and one wrinkled green as depicted in the Punnett square, named after the British mathematician who introduced it (in the lower part of Fig. 1-3). This **principle of independent** assortment is frequently called Mendel's second law.

CHROMOSOMAL THEORY OF HEREDITY

A principal reason for the original failure to appreciate Mendel's discovery was the absence of firm facts about the behavior of chromosomes during meiosis and mitosis. This knowledge was available, however, when Mendel's laws were confirmed in 1900 and was seized upon in 1903 by American biologist Walter S. Sutton. In his classic paper "The Chromosomes in Heredity," Sutton emphasized the importance of the fact that the diploid chromosome group consists of two morphologically similar sets and that, during meiosis, every gamete receives only one chromosome of each homologous pair. He then used this fact to explain Mendel's results by assuming that genes are parts of the chromosome. He postulated that the yellow- and green-seed genes are carried on a certain pair of chromosomes and that the round- and wrinkled-seed genes are carried on a different pair. This hypothesis immediately explains the experimentally observed 9:3:3:1 segregation ratios. Although Sutton's paper did not prove the chromosomal theory of heredity, it was immensely important, for it brought together for the first time the independent disciplines of genetics (the study of breeding experiments) and cytology (the study of cell structure).

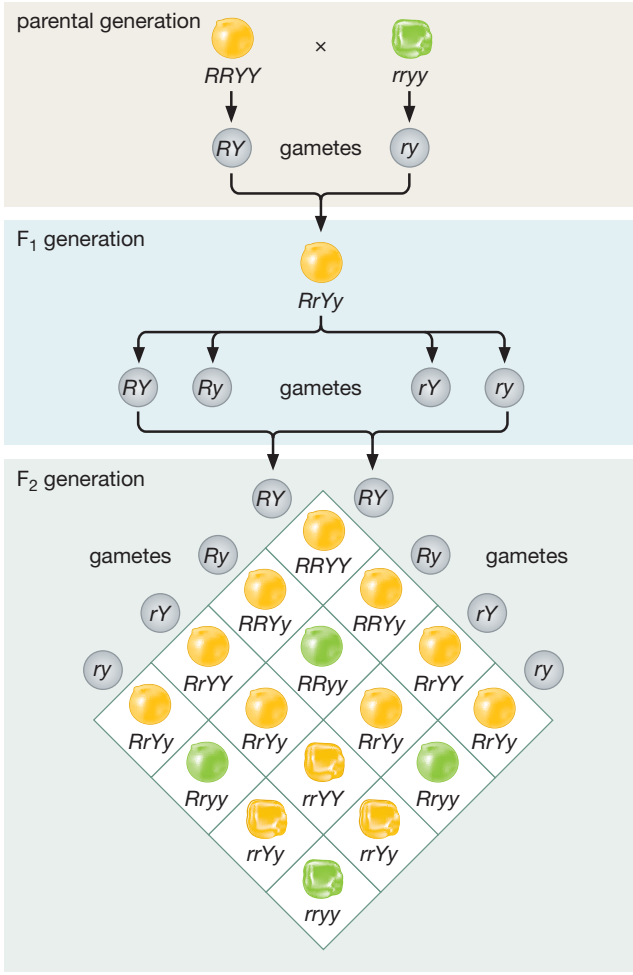


FIGURE 1-3 How Mendel's second law (independent assortment) operates. In this example, the inheritance of yellow (*Y*) and green (*y*) seed color is followed together with the inheritance of round (*R*) and wrinkled (*r*) seed shapes. The *R* and *Y* alleles are dominant over *r* and *y*. The genotypes of the various parents and progeny are indicated by letter combinations, and four different phenotypes are distinguished by appropriate shading.

GENE LINKAGE AND CROSSING OVER

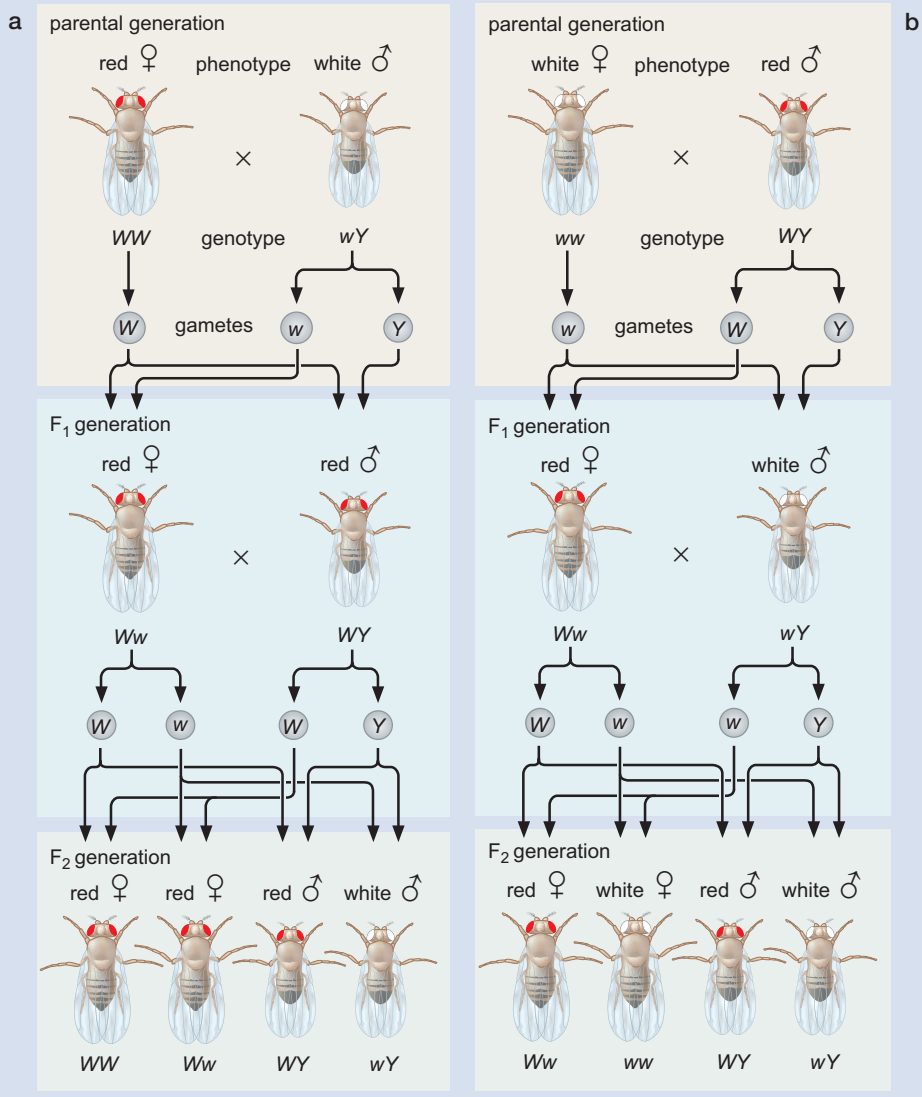
Mendel's principle of independent assortment is based on the fact that genes located on different chromosomes behave independently during meiosis. Often, however, two genes do not assort independently because they are located on the same chromosome (**linked genes**; see Box 1-2, Genes Are Linked to Chromosomes). Many examples of nonrandom assortment were found as soon as a large number of mutant genes became available for breeding analysis. In every well-studied case, the number of linked groups was identical to the haploid chromosome number. For example, there are four groups of linked genes in *Drosophila* and four morphologically distinct chromosomes in a haploid cell.

Linkage, however, is in effect never complete. The probability that two genes on the same chromosome will remain together during meiosis ranges from just less than 100% to nearly 50%. This variation in linkage suggests that there must be a mechanism for exchanging genes on homologous chromosomes. This mechanism is called **crossing over**. Its cytological basis was first described by Belgian cytologist F.A. Janssens. At the start of meiosis, through the process of **synapsis**, the homologous chromosomes form pairs with their long axes parallel. At this stage, each chromosome has duplicated to form two chromatids. Thus, synapsis brings together four chromatids (a tetrad), which coil about one another. Janssens postulated that, possibly

Box 1-2 Genes Are Linked to Chromosomes

Initially, all breeding experiments used genetic differences already existing in nature. For example, Mendel used seeds obtained from seed dealers, who must have obtained them from farmers. The existence of alternative forms of the same gene (alleles) raises the question of how they arose. One obvious hypothesis states that genes can change (mutate) to give rise to new genes (**mutant genes**). This hypothesis was first seriously tested, beginning in 1908, by the great American biologist Thomas Hunt Morgan and his young collaborators, geneticists Calvin B. Bridges, Hermann J. Muller, and Alfred H. Sturtevant. They worked with the tiny fly *Drosophila melanogaster*. The first mutant found was a male with white eyes instead of the normal red eyes. The white-eyed variant appeared spontaneously in a culture bottle of red-eyed flies. Because essentially all *Drosophila* found in nature have red eyes, the gene leading to red eyes was referred to as the **wild-type gene**; the gene leading to white eyes was called a mutant gene (allele).

The white-eye mutant gene was immediately used in breeding experiments (Box 1-2 Fig. 1), with the striking result that the behavior of the allele completely paralleled the distribution of an X chromosome (i.e., was sex-linked). This finding immediately suggested that this gene might be located on the X chromosome, together with those genes controlling sex. This hypothesis was quickly confirmed by additional genetic crosses using newly isolated mutant genes. Many of these additional mutant genes also were sex-linked.



BOX 1-2 FIGURE 1 The inheritance of a sex-linked gene in *Drosophila*. Genes located on sex chromosomes can express themselves differently in male and female progeny, because if there is only one *X* chromosome present, recessive genes on this chromosome are always expressed. Here are two crosses, both involving a recessive gene (*w*, for white eye) located on the *X* chromosome. (a) The male parent is a white-eyed (*wY*) fly, and the female is homozygous for red eye (*WW*). (b) The male has red eyes (*WY*) and the female white eyes (*ww*). The letter *Y* stands here not for an allele, but for the *Y* chromosome, present in male *Drosophila* in place of a homologous *X* chromosome. There is no gene on the *Y* chromosome corresponding to the *w* or *W* gene on the *X* chromosome.

because of tension resulting from this coiling, two of the chromatids might sometimes break at a corresponding place on each. These events could create four broken ends, which might rejoin crossways, so that a section of each of the two chromatids would be joined to a section of the other (Fig. 1-4). In this manner, recombinant chromatids might be produced that contain a segment derived from each of the original homologous chromosomes. Formal proof of Janssens's hypothesis that chromosomes physically interchange material during synapsis came more than 20 years later, when in 1931, Barbara McClintock and Harriet B. Creighton, working at Cornell University with the corn plant *Zea mays*, devised an elegant cytological demonstration of chromosome breakage and rejoining (Fig. 1-5).

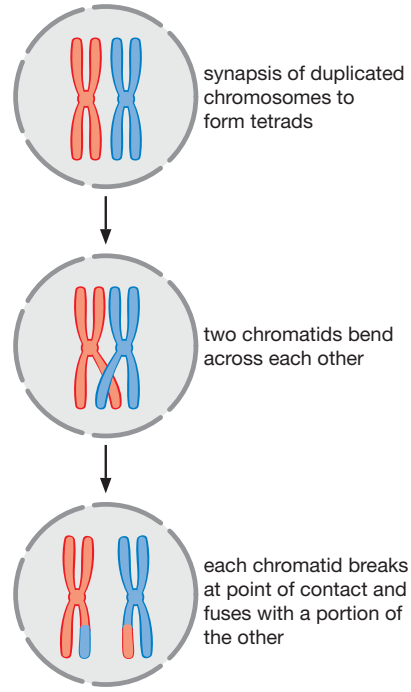


FIGURE 1-4 Janssens's hypothesis of crossing over.

CHROMOSOME MAPPING

Thomas Hunt Morgan and his students, however, did not await formal cytological proof of crossing over before exploiting the implication of Janssens's hypothesis. They reasoned that genes located close together on a chromosome would assort with one another much more regularly (close linkage) than genes located far apart on a chromosome. They immediately saw this as a way to locate (map) the relative positions of genes on chromosomes and thus to produce a **genetic map**. The way they used the frequencies of the various recombinant classes is very straightforward. Consider the segregation of three genes all located on the same chromosome. The arrangement of the genes can be determined by means of three crosses, in each of which two genes are followed (two-factor crosses). A cross between *AB* and *ab* yields four progeny types: the two parental genotypes (*AB* and *ab*) and two recombinant genotypes (*Ab* and *aB*). A cross between *AC* and *ac* similarly gives two parental combinations as well as the *Ac* and *aC*

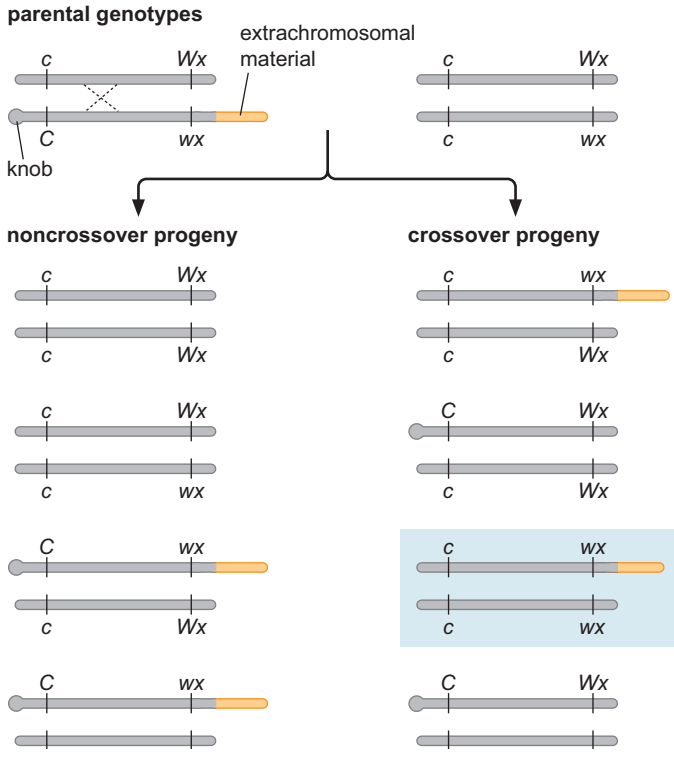


FIGURE 1-5 Demonstration of physical exchanges between homologous chromosomes. In most organisms, pairs of homologous chromosomes have identical shapes. Occasionally, however, the two members of a pair are not identical; one is marked by the presence of extrachromosomal material or compacted regions that reproducibly form knob-like structures. McClintock and Creighton found one such pair and used it to show that crossing over involves actual physical exchanges between the paired chromosomes. In the experiment shown here, the homozygous *c, wx* progeny had to arise by crossing over between the *C* and *wx* loci. When such *c, wx* offspring were cytologically examined, knob chromosomes were seen, showing that a knobless *Wx* region had been physically replaced by a knobbed *wx* region. The colored box in the figure identifies the chromosomes of the homozygous *c, wx* offspring.

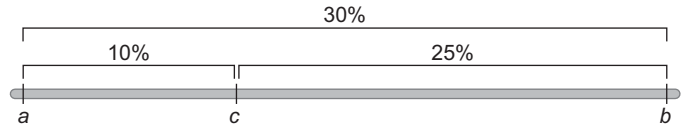


FIGURE 1-6 **Assignment of the tentative order of three genes on the basis of three two-factor crosses.**

recombinants, whereas a cross between *BC* and *bc* produces the parental types and the recombinants *Bc* and *bC*. Each cross will produce a specific ratio of parental to recombinant progeny. Consider, for example, the fact that the first cross gives 30% recombinants, the second cross 10%, and the third cross 25%. This tells us that genes *a* and *c* are closer together than *a* and *b* or *b* and *c* and that the genetic distances between *a* and *b* and *b* and *c* are more similar. The gene arrangement that best fits these data is *a-c-b* (Fig. 1-6).

The correctness of gene order suggested by crosses of two gene factors can usually be unambiguously confirmed by three-factor crosses. When the three genes used in the preceding example are followed in the cross *ABC* × *abc*, six recombinant genotypes are found (Fig. 1-7). They fall into three groups of reciprocal pairs. The rarest of these groups arises from a double crossover. By looking for the least frequent class, it is often possible to instantly confirm (or deny) a postulated arrangement. The results in Figure 1-7 immediately confirm the order hinted at by the two-factor crosses. Only if the order is *a-c-b* does the fact that the rare recombinants are *AcB* and *aCb* make sense.

The existence of multiple crossovers means that the amount of recombination between the outside markers *a* and *b* (*ab*) *is* usually less than the sum of the recombination frequencies between *a* and *c* (*ac*) and *c* and *b* (*cb*). To obtain a more accurate approximation of the distance between the outside markers, we calculate the probability (*ac* × *cb*) that when a crossover occurs between *c* and *b*, a crossover also occurs between *a* and *c*, and vice versa (*cb* × *ac*). This probability subtracted from the sum of the frequencies expresses more accurately the amount of recombination. The simple formula

$$ab = ac + cb - 2(ac)(cb)$$

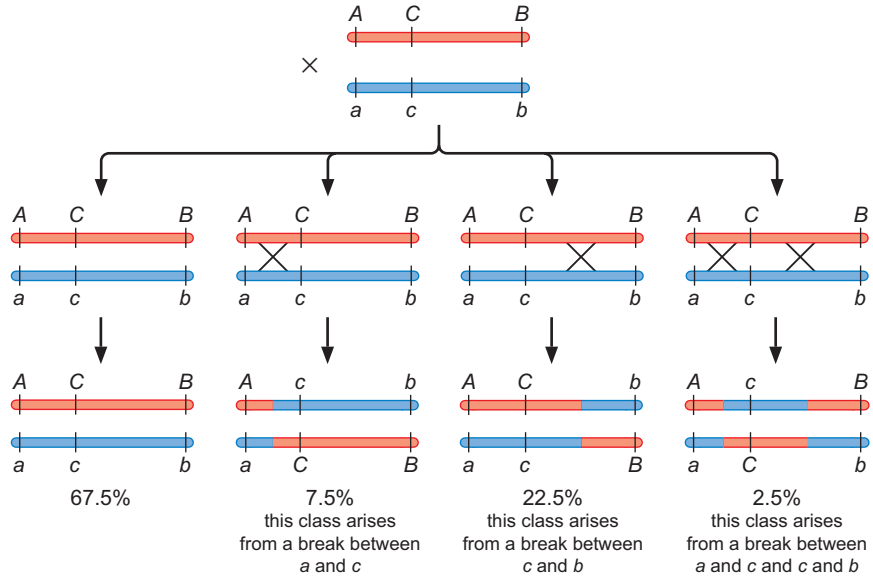


FIGURE 1-7 **The use of three-factor crosses to assign gene order.** The least frequent pair of reciprocal recombinants must arise from a double crossover. The percentages listed for the various classes are the theoretical values expected for an infinitely large sample. When finite numbers of progeny are recorded, the exact values will be subject to random statistical fluctuations.

is applicable in all cases where the occurrence of one crossover does not affect the probability of another crossover. Unfortunately, accurate mapping is often disturbed by *interference* phenomena, which can either increase or decrease the probability of correlated crossovers.

Using such reasoning, the Columbia University group headed by Morgan had by 1915 assigned locations to more than 85 mutant genes in *Drosophila* (Table 1-1), placing each of them at distinct spots on one of the four linkage groups, or chromosomes. Most importantly, all the genes on a given chromosome were located on a line. The gene arrangement was strictly linear and never branched. The genetic map of one of the chromosomes of *Drosophila* is shown in Figure 1-8. Distances between genes on such a map are measured in **map units**, which are related to the frequency of recombination between the genes. Thus, if the frequency of recombination between two genes is found to be 5%, the genes are said to be separated by five map units. Because of the high probability of double crossovers between widely spaced genes, such assignments of map units can be considered accurate only if recombination between closely spaced genes is followed.

Even when two genes are at the far ends of a very long chromosome, they assort together at least 50% of the time because of multiple crossovers. The two genes will be separated if an odd number of crossovers occurs between them, but they will end up together if an even number occurs between them. Thus, in the beginning of the genetic analysis of *Drosophila*, it was often impossible to determine whether two genes were on different chromosomes or at the opposite ends of one long chromosome. Only after large numbers of genes had been mapped was it possible to demonstrate convincingly that the number of linkage groups equalled the number of cytologically visible chromosomes. In 1915, Morgan, with his students Alfred H. Sturtevant, Hermann J. Muller, and Calvin B. Bridges, published their definitive book *The Mechanism of Mendelian Heredity*, which first announced the general validity of the chromosomal basis of heredity. We now rank this concept, along with the theories of evolution and the cell, as a major achievement in our quest to understand the nature of the living world.

THE ORIGIN OF GENETIC VARIABILITY THROUGH MUTATIONS

It now became possible to understand the hereditary variation that is found throughout the biological world and that forms the basis of the theory of evolution. Genes are normally copied exactly during chromosome duplication. Rarely, however, changes (**mutations**) occur in genes to give rise to altered forms, most—*but not all*—of which function less well than the wild-type alleles. This process is necessarily rare; otherwise, many genes would be changed during every cell cycle, and offspring would not ordinarily resemble their parents. There is, instead, a strong advantage in there being a small but finite mutation rate; it provides a constant source of new variability, necessary to allow plants and animals to adapt to a constantly changing physical and biological environment.

Surprisingly, however, the results of the Mendelian geneticists were not avidly seized upon by the classical biologists, then the authorities on the evolutionary relations between the various forms of life. Doubts were raised about whether genetic changes of the type studied by Morgan and his students were sufficient to permit the evolution of radically new structures,

TABLE 1-1 The 85 Mutant Genes Reported in *Drosophila melanogaster* in 1915

Name	Region Affected	Name	Region Affected
Group 1			
Abnormal	Abdomen	*Lethal, 13*	Body, death
Bar	Eye	*Miniature*	Wing
Bifid	Venation	*Notch*	Venation
Bow	Wing	*Reduplicated*	Eye color
Cherry	Eye color	*Ruby*	Leg
Chrome	Body color	*Rudimentary*	Wing
Cleft	Venation	*Sable*	Body color
Club	Wing	*Shifted*	Venation
Depressed	Wing	*Short*	Wing
Dotted	Thorax	*Skee*	Wing
Eosin	Eye color	*Spoon*	Wing
Facet	Ommatidia	*Spot*	Body color
Forked	Spine	*Tan*	Antenna
Furrowed	Eye	*Truncate*	Wing
Fused	Venation	*Vermilion*	Eye color
Green	Body color	*White*	Eye color
Jaunty	Wing	*Yellow*	Body color
Lemon	Body color		
Group 2			
Antlered	Wing	*Jaunty*	Wing
Apterous	Wing	*Limited*	Abdominal band
Arc	Wing	*Little crossover*	Chromosome 2
Balloon	Venation	*Morula*	Ommatidia
Black	Body color	*Olive*	Body color
Blistered	Wing	*Plexus*	Venation
Comma	Thorax mark	*Purple*	Eye color
Confluent	Venation	*Speck*	Thorax mark
Cream II	Eye color	*Strap*	Wing
Curved	Wing	*Streak*	Pattern
Dachs	Leg	*Trefoil*	Pattern
Extra vein	Venation	*Truncate*	Wing
Fringed	Wing	*Vestigial*	Wing
Group 3			
Band	Pattern	*Pink*	Eye color
Beaded	Wing	*Rough*	Eye
Cream III	Eye color	*Safranin*	Eye color
Deformed	Eye	*Sepia*	Eye color
Dwarf	Size of body	*Sooty*	Body color
Ebony	Body color	*Spineless*	Spine
Giant	Size of body	*Spread*	Wing
Kidney	Eye	*Trident*	Pattern
Low crossing over	Chromosome 3	*Truncate*	Wing
Maroon	Eye color	*Whitehead*	Pattern
Peach	Eye color	*White ocelli*	Simple eye
Group 4			
Bent	Wing	*Eyeless*	Eye

The mutations fall into four linkage groups. Because four chromosomes were cytologically observed, this indicated that the genes are situated on the chromosomes. Notice that mutations in various genes can act to alter a single character, such as body color, in different ways.

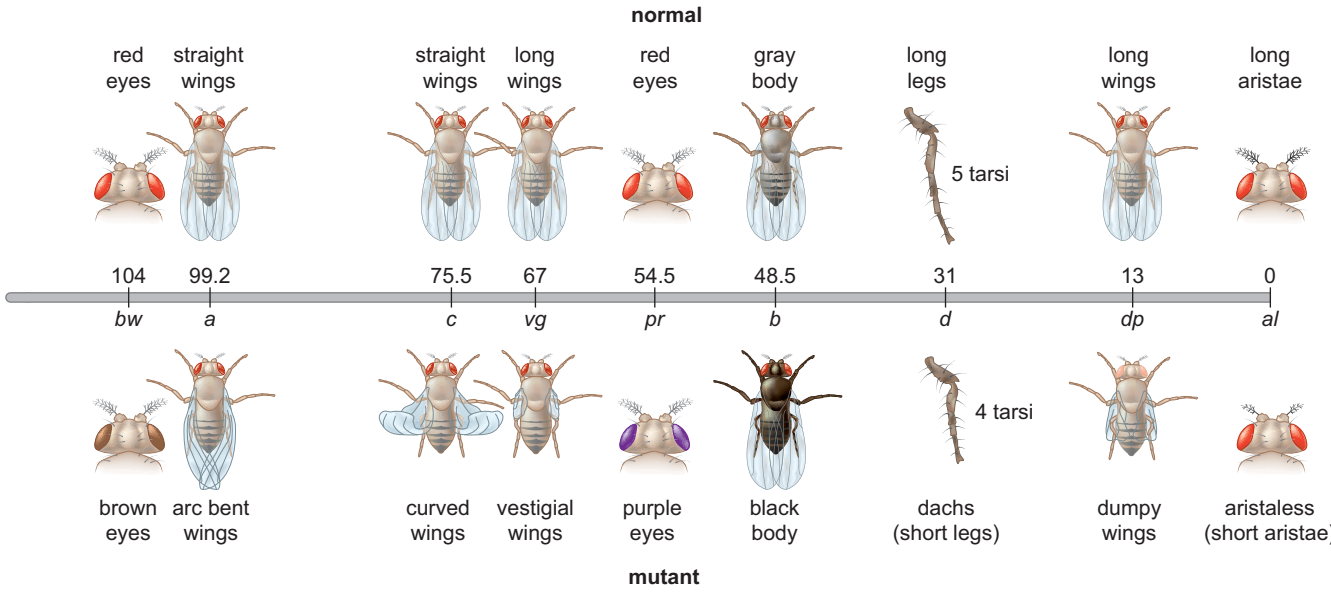


FIGURE 1-8 **The genetic map of chromosome 2 of *Drosophila melanogaster*.**

like wings or eyes. Instead, these biologists believed that there must also occur more powerful "macromutations," and that it was these events that allowed great evolutionary advances.

Gradually, however, doubts vanished, largely as a result of the efforts of the mathematical geneticists Sewall Wright, Ronald A. Fisher, and John Burden Sanderson Haldane. They showed that, considering the great age of Earth, the relatively low mutation rates found for *Drosophila* genes, together with only mild selective advantages, would be sufficient to allow the gradual accumulation of new favorable attributes. By the 1930s, biologists began to reevaluate their knowledge of the origin of species and to understand the work of the mathematical geneticists. Among these new Darwinians were biologist Julian Huxley (a grandson of Darwin's original publicist, Thomas Huxley), geneticist Theodosius Dobzhansky, paleontologist George Gaylord Simpson, and ornithologist Ernst Mayr. In the 1940s all four wrote major works, each showing from his special viewpoint how Mendelianism and Darwinism were indeed compatible.

EARLY SPECULATIONS ABOUT WHAT GENES ARE AND HOW THEY ACT

Almost immediately after the rediscovery of Mendel's laws, geneticists began to speculate about both the chemical structure of the gene and the way it acts. No real progress could be made, however, because the chemical identity of the genetic material remained unknown. Even the realization that both nucleic acids and proteins are present in chromosomes did not really help, since the structure of neither was at all understood. The most fruitful speculations focused attention on the fact that genes must be, in some sense, self-duplicating. Their structure must be exactly copied every time one chromosome becomes two. This fact immediately raised the profound chemical question of how a complicated molecule could be precisely copied to yield exact replicas.

Some physicists also became intrigued with the gene, and when quantum mechanics burst on the scene in the late 1920s, the possibility arose that in order to understand the gene, it would first be necessary to master the subtleties of the most advanced theoretical physics. Such thoughts, however, never really took root, since it was obvious that even the best physicists or theoretical chemists would not concern themselves with a substance whose structure still awaited elucidation. There was only one fact that they might ponder: Muller's and L.J. Stadler's independent 1927 discoveries that X-rays induce mutations. Because there is a greater possibility that an X-ray will hit a larger gene than a smaller gene, the frequency of mutations induced in a given gene by a given X-ray dose yields an estimate of the size of this gene. But even here, so many special assumptions were required that virtually no one, not even Muller and Stadler themselves, took the estimates very seriously.

PRELIMINARY ATTEMPTS TO FIND A GENE–PROTEIN RELATIONSHIP

The most fruitful early endeavors to find a relationship between genes and proteins examined the ways in which gene changes affect which proteins are present in the cell. At first these studies were difficult, because no one knew anything about the proteins that were present in structures such as the eye or the wing. It soon became clear that genes with simple metabolic functions would be easier to study than genes affecting gross structures. One of the first useful examples came from a study of a hereditary disease affecting amino acid metabolism. Spontaneous mutations occur in humans affecting the ability to metabolize the amino acid phenylalanine. When individuals homozygous for the mutant trait eat food containing phenylalanine, their inability to convert the amino acid to tyrosine causes a toxic level of phenylpyruvic acid to build up in the bloodstream. Such diseases, examples of "inborn errors of metabolism," suggested to English physician Archibald E. Garrod, as early as 1909, that the wild-type gene is responsible for the presence of a particular enzyme, and that in a homozygous mutant, the enzyme is congenitally absent.

Garrod's general hypothesis of a gene–enzyme relationship was extended in the 1930s by work on flower pigments by Haldane and Rose Scott-Moncrieff in England, studies on the hair pigment of the guinea pig by Wright in the United States, and research on the pigments of insect eyes by A. Kuhn in Germany and by Boris Ephrussi and George W. Beadle, working first in France and then in California. In all cases, the evidence revealed that a particular gene affected a particular step in the formation of the respective pigment whose absence changed, say, the color of a fly's eyes from red to ruby. However, the lack of fundamental knowledge about the structures of the relevant enzymes ruled out deeper examination of the gene–enzyme relationship, and no assurance could be given either that most genes control the synthesis of proteins (by then it was suspected that all enzymes were proteins) or that all proteins are under gene control.

As early as 1936, it became apparent to the Mendelian geneticists that future experiments of the sort successful in elucidating the basic features of Mendelian genetics were unlikely to yield productive evidence about how genes act. Instead, it would be necessary to find biological objects more suitable for chemical analysis. They were aware, moreover, that contemporary knowledge of nucleic acid and protein chemistry was completely inadequate for a fundamental chemical attack on even the most suitable

biological systems. Fortunately, however, the limitations in chemistry did not deter them from learning how to do genetic experiments with chemically simple molds, bacteria, and viruses. As we shall see, the necessary chemical facts became available almost as soon as the geneticists were ready to use them.

SUMMARY

Heredity is controlled by chromosomes, which are the cellular carriers of genes. Hereditary factors were first discovered and described by Mendel in 1865, but their importance was not realized until the start of the 20th century. Each gene can exist in a variety of different forms called alleles. Mendel proposed that a hereditary factor (now known to be a gene) for each hereditary trait is given by each parent to each of its offspring. The physical basis for this behavior is the distribution of homologous chromosomes during meiosis: one (randomly chosen) of each pair of homologous chromosomes is distributed to each haploid cell. When two genes are on the same chromosome, they tend to be inherited together (linked). Genes affecting different characteristics are sometimes inherited independently of each other, because they are located on different chromosomes. In any case, linkage is seldom complete because homologous chromosomes attach to each other during meiosis and often break at identical spots and rejoin crossways (crossing over). Crossing over transfers genes initially located on a paternally derived chromosome onto gene groups originating from the maternal parent.

Different alleles from the same gene arise by inheritable changes (mutations) in the gene itself. Normally, genes are extremely stable and are copied exactly during chromosome duplication; mutation occurs only rarely and usually has harmful consequences. Mutation does, however, play a positive role, because the accumulation of rare favorable mutations provides the basis for genetic variability that is presupposed by the theory of evolution.

For many years, the structure of genes and the chemical ways in which they control cellular characteristics were a mystery. As soon as large numbers of spontaneous mutations had been described, it became obvious that a one gene–one characteristic relationship does not exist and that all complex characteristics are under the control of many genes. The most sensible idea, postulated by Garrod in 1909, was that genes affect the synthesis of enzymes. However, the tools of Mendelian geneticists—organisms such as the corn plant, the mouse, and even the fruit fly *Drosophila*—were not suitable for detailed chemical investigations of gene–protein relations. For this type of analysis, work with much simpler organisms was to become indispensable.

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QUESTIONS

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For answers to even-numbered questions, see Appendix 2: Answers.

Question 1. You are comparing two alleles of Gene *X*. What defines the two alleles as distinct alleles?

Question 2. True or false. Explain your choice. One gene possesses only two alleles.

Question 3. True or false. Explain your choice. One trait is always determined by one gene.

Question 4. True or false. Explain your choice. For a given gene, one can always define the alleles as dominant or recessive.

Question 5. You want to identify dominant/recessive relationship for skin color for a new frog species that you found in the rain forest. Assume that one autosomal gene controls skin color in this species. All of the frogs that you found for that species are bright blue or yellow. A bright blue female and bright blue male frog mate and produce all bright blue progeny. A yellow female and yellow male frog mate and produce a mix of bright blue and yellow progeny. Identify each trait (bright blue skin color and yellow skin color) as dominant or recessive. Explain your choices. Identify the genotype for each parent in the two crosses. Use the letter *B* to refer to the gene conferring skin color.

Question 6.

A. After crossing true-breeding pea plants with yellow seeds to true-breeding pea plants with green seeds as Mendel did, what phenotype do you expect for the pea plants in the F_1 generation if yellow seeds are dominant to green seeds?

B. You self-cross the F_1 generation. Give the expected phenotypic ratio of the F_2 generation.

C. Give the expected genotypic ratio of the F_2 generation.

D. Give the expected ratio of heterozygotes to homozygotes in the F_2 generation.

Question 7. Mendel studied seven distinct traits for pea plants. By luck six of the traits were on different chromosomes, and two traits were separated by a great distance on one chromosome. If Mendel selected two traits controlled by linked genes in his initial studies, which law would be affected (Mendel's first or second law)? Explain your choice.

Question 8. You want to map the positions of three genes (*X*, *Y*, and *Z*) all found on one chromosome in *Drosophila*. Each gene has one dominant allele and one recessive allele. You perform the three different two-factor crosses (Cross 1: *XY* and *xy*, Cross 2: *YZ* and *yz,* and Cross 3: *XZ* and *xz*). Assume all crosses are between diploid flies homozygous for the alleles of these genes. You observe 7% recombinants in the first cross, 20% recombinants in the second cross, and 13% recombinants in the third cross. Draw a map placing the genes in the proper order and give the distance between each gene in map units (m.u.).

Question 9. You want to confirm your ordering for Question 8 using a three-factor cross (cross *XYZ*/*xyz* and *xyz*/*xyz*). Your least common recombinants are *xYZ* and *Xyz*. Does this confirm your order from Question 8? Explain why or why not.

Question 10. You again want to map the positions of three genes (*L*, *M*, and *N)* in *Drosophila*. Each gene has one dominant allele and one recessive allele. You perform the three different two-factor crosses (Cross 1: *LM* and *lm*, Cross 2: *MN* and *mn,* and Cross 3: *LN* and *ln*). Assume all crosses are between diploid flies homozygous for the alleles of these genes. You observe 5% recombinants in the first cross, 50% recombinants in the second cross, and 50% recombinants in the third cross. Based on the data given, what can you determine for the gene order and distance between the genes?

Question 11. Following up on the observations in Question 10, you complete new crosses using gene *O*. You observe 30% recombination for a cross between *MO* and *mo*, 35% recombination for a cross between *LO* and *lo*, and 25% recombination for a cross between *NO* and *no*. Assume all crosses are between diploid flies homozygous for the alleles of these genes. Given the information from Questions 10 and 11, draw a map placing the genes in the proper order and give the distance between each gene in map units.

Question 12. Define mutation. The cell has many mechanisms to prevent mutations. Explain how a very low mutation rate could be advantageous over the prevention of all mutations in an organism.

Question 13. Differentiate between chromosomes and chromatids.

Question 14. You are mapping the 6th chromosome of the sheep blowfly *Lucilia cuprina* and want to test how your calculations compare to a published map. In a recent cross, you studied the mutations *tri*, *pk*, and *y* that display thickened vein junctions, pink body color, and yellow eyes, respectively. From a cross between a male homozygous for the three mutations and a heterozygous female (*tri pk y*/+++), you record the counts for the progeny. In the published map, the distance between *y* and *pk* is 23.0 m.u., the distance between *pk* and *tri* is 18.4 m.u., and the distance between *y* and *tri* is 41.4 m.u. Based on the published map and given values below, calculate the expected values for observed progeny that represent either a single or double crossover. Remember that your observed values are data that include some statistical fluctuations.

Total progeny counted: 1000
Total recombinants that represent a double crossover: 15

Published map information from Weller and Foster (1993. *Genome* **36:** 495–506).

Question 15. You are studying a new species of bird. You know the species has sex chromosomes similar to chicken. Males carry two Z chromosomes, whereas females carry one Z chromosome and one W chromosome. Because the genome has not been sequenced yet, you will perform crosses to gain more genetic information. You are interested in the eye color of the birds. You obtain true-breeding birds with black or green eyes. You cross a black-eyed male to a green-eyed female. Assume the trait is determined by one gene.

A. Considering a dominant/recessive relationship, you want to determine if black is recessive to green or if green is recessive to black. How could you use the phenotypes for the F_1 and F_2 progeny to help you answer this question?

B. If the trait is sex-linked, refine your answer to part A with respect to the dominant/recessive relationship of a sex-linked trait on the Z chromosome for the F_1 generation.

C. Assume that black is dominant to green. You cross a black-eyed male from the F_1 generation to a black-eyed female from the F_1 generation. If the trait is sex-linked, predict the genotypic and phenotypic ratios for the F_2 generation.