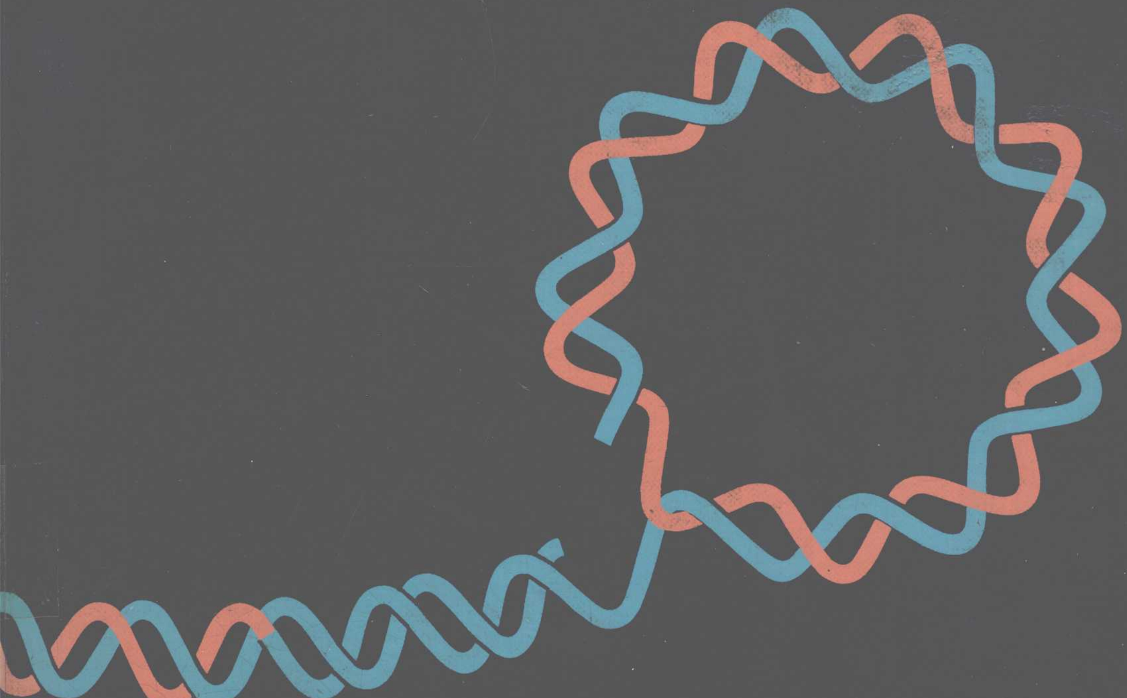
SECOND EDITION

MOLECULAR GENETICS

AN INTRODUCTORY NARRATIVE

Gunther S. Stent
Richard Calendar



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UNIVERSITY OF CALIFORNIA, BERKELEY

W. H. FREEMAN AND COMPANY
San Francisco

FRONTISPIECE

Direct visualization of RNA transcription of genes. An electron micrograph of a portion of an extrachromosomal nucleolus isolated from an immature egg cell (oocyte) of a newt. Bottle-brush-like matrix elements (M) separated by matrix-free segments of the core axis (A) can be seen. The core axis is a protein-covered DNA double helix, and the thin fibrils of the matrix elements lying perpendicular to the core axis are protein-covered molecules of nascent ribosomal RNA being transcribed by RNA polymerase from the DNA template. The DNA core of each matrix consists of a gene carrying the nucleotide sequence of the ribosomal RNA, whose transcription evidently proceeds in the direction of lengthening perpendicular RNA fibrils. About a hundred RNA molecules are being transcribed simultaneously from a single gene. [From O. L. Miller and B. R. Beatty, *Science* **164**, 956 (1969). Copyright 1969 by American Association for the Advancement of Science.]

Library of Congress Cataloging in Publication Data
Stent, Gunther Siegmund, 1924–
Molecular genetics.

Includes bibliographies and index.
1. Molecular genetics. I. Calendar, Richard, joint author. II. Title.
QH430.S73 1978 574.8′732 78-688
ISBN 0-7167-0048-4

Printed in the United States of America

9 8 7 6 5 4 3

Preface to the First Edition

In the winter of 1954, Edward H. Adelberg and I began teaching an undergraduate course at the University of California that was supposed to bring the latter-day gospel of molecular genetics to the Berkeley students. It was an extraordinarily gratifying pedagogical undertaking to face an audience of innocents, who had not yet heard of the DNA double helix, and preach to them that a new era was dawning for the understanding of heredity. So enthusiastic were we in those days that we managed to give thirty lectures on what comparatively little was then known about mutation and genetic recombination in bacteria and their viruses. How times have changed! Molecular genetics has since grown from the esoteric specialty of a small, tightly knit vanguard into an elephantine academic discipline whose basic doctrines today form part of the primary-school science curriculum. Throughout the period of the well-nigh explosive development of its subject, I have continued to teach this course, and had I not undertaken an annual pruning of the material, the number of lectures necessary to present it would by now have grown at least tenfold. This text presents the present scope and content of that course.

The evolutionary origin and essentially pedagogic purpose of this book are reflected in the narrative presentation of the material in the historical sequence in which it actually came to be known (and in the occasional burdening of the reader with long-abandoned theories). Not only did the text simply grow in this way, but also I happen to believe that an understanding of the essentials of molecular genetics can best be taught in an organic (rather than logical) manner. But in case my presentation should give the erroneous impression that it is an attempt at historiography, I must warn the reader that my "streamlined" account of past developments is intended to be neither a scholarly history nor a hagiography of molecular genetics.

In the first place, I have deliberately chosen to mention only a part of the diverse experimental materials that actually figured in the development of my subject. Without doing too much violence to what I believe to have been the actual sequence of events, I have for the most part tried to present the basic findings of molecular genetics as they came to be revealed through work done with the hemoglobins of humans and rabbits, with the tryptophan synthetase, the betagalactosidase, and the DNA-, RNA-, and protein-synthesizing machinery of the intestinal bacterium *Escherichia coli*, and with three or four viruses that grow on *E. coli*. Thus there are vast lacunae in my account: for instance, the two eukaryotic microbes that were the subjects of much intensive molecular genetic study, the bread mold *Neurospora crassa* and the yeast *Saccharomyces*, are mentioned only in passing; the bacterial enzymes active in the synthesis of arginine, histidine, and leucine and in the hydrolysis of organic phosphates, whose study contributed in a very important way to the understanding of the mechanics and regulation of protein synthesis, are given short shrift; and animal viruses, the study of whose reproduction paid enormous benefits in both practical and theoretical realms, have been passed over in near total silence.

In the second place my story is historiographically defective as far as its mentioning of persons' names is concerned. The many investigators who happen to have worked with experimental material that I did not choose for presentation are completely missing from this account. Even the names of most of those who labored to bring forth the body of knowledge on which I *do* report have been left unmentioned, for I feared that providing a complete dramatis personae would prove tiresome for my readers. But neither did I want to opt for the other alternative of equitable scientific historiography—if you don't cite everybody, cite nobody—since I thought that every student ought to know the identity of at least *some* of the protagonists of the theater of molecular genetics. And so I have made an undoubtedly invidious selection of names, among which my own friends are probably over-represented.

I have attempted to present my material in such a manner that it is within the intellectual horizon of a reader who has completed two years of undergraduate science training. The only formal preparation that I have taken for granted is a year's study of general college chemistry, so that I am presuming at least a superficial familiarity with such concepts as atoms and molecules, weak and strong chemical bonds, chemical equilibrium, and oxidation-reduction and solution chemistry. I am not presuming prior college study in biology, and particularly not in genetics, although a command of these subjects, as well as of organic chemistry, should certainly help in the understanding of this text. Unfortunately, the undertaking to make the story of molecular genetics accessible to such a broad audience entailed the unavoidable drawback that some of the material presented here must be familiar to readers with prior college training in the life sciences. Although in composing my narrative I did have in mind a devoted reader who sticks with it from beginning to end, most veterans of a modern general biology course might prefer to begin their study of this text with Chapter 3, and readers in possession of the basic facts and terminology of biochemistry might even proceed directly to Chapter 5.

In order to open avenues to further and deeper study of molecular genetics, I have provided each chapter with a bibliography of pertinent literature. These bibliographies include three categories of references. The first category indicates the relevant chapters of three other books, which between them cover in greater detail much of the ground of this text. One is the collection of autobiographical and retrospective essays *Phage and the Origins of Molecular Biology* (J. Cairns, G. S. Stent, and J. D. Watson, eds., Cold Spring Harbor Laboratory of Quantitative Biology, New York, 1966). These essays, which were written by members of the Phage Group of which Max Delbrück was the central figure, trace out the intellectual, experimental, and personal developments that led to some of the main happenings of this story. The other two books are William Hayes' definitive text, *The Genetics of Bacteria and Their Viruses* (2nd edition, John Wiley & Sons, New York, 1968), and my own earlier text, *Molecular Biology of Bacterial Viruses* (W. H. Freeman and Company, San Francisco, 1963). These books are abbreviated as HAYES and MOBIBAV, respectively, in the bibliographies. The second category includes references to some of the original research papers in which the results of key experiments were first reported. The third category includes specialized texts, reviews, and monographs, in which the reader can find both further information and more extensive bibliographies.

Finally, I ought to mention here an outstanding book that covers more or less the same ground as this text: J. D. Watson's *Molecular Biology of the Gene* (2nd edition, W. A. Benjamin, New York, 1970). I have the highest regard for this deservedly successful introductory presentation; in my opinion, it has no peer in the literature of molecular genetics. Indeed, the only reason why I persevered in completing my own treatment of the same material is that I thought that some readers might profit more from my narrative approach than from Watson's sovereign didactics. But it is according to the high standards set by *Molecular Biology of the Gene* that I wish my own effort to be judged.

This text was completed during a sabbatical leave from the University of California, Berkeley. I thank the John Simon Guggenheim Memorial Foundation for its grant of a fellowship and Stephen W. Kuffler and John Nicholls of the Department of Neurobiology, Harvard Medical School, for hospitable accommodation in their laboratories. I am grateful to Robert S. Edgar, A. Dale Kaiser, and Charles Yanofsky for their critical readings of the manuscript and to Mrs. Margery Hoogs for her efforts to rectify its prose style.

August 1970 *Gunther S. Stent*

FIGURE 1-1
Gregor Mendel (1822–1884). [Courtesy of the Moravian Museum, Brno.]

Contents

12 Genetic Fine Structure 375

13 Lysogeny and Transduction 413

14 DNA Transcription 464

15 RNA Translation 501

16 Genetic Code 540

17 Genetic RNA 575

1

Heredity

GREGOR MENDEL

One summer evening in 1965, an enormous crowd, probably one of the largest in its 600-year history, packed the Church of the Assumption in the Moravian town of Brno to celebrate a memorial Mass for Gregor Mendel (Figure 1-1), one-time abbot of the Augustinian monastery to which that church had formerly belonged. It was not so much piety for a departed prelate that had brought together that largely noncommunicant crowd, but rather the wish to pay homage to the memory of the founder of genetics. For in Mendel's own church was then gathered an ecumenical body of geneticists who had come to Brno from all parts of the world at the invitation of the Czechoslovak Academy of Sciences to commemorate the 100th anniversary of the presentation of Mendel's paper "Experiments on Plant Hybrids," which in 1865 was reported to the Brno Society of Natural Science. This rite, in view of its timing, locale, and auspices, served also as a Te Deum for the official resurrection of genetics and for the rehabilitation of those geneticists who had survived nearly two decades of official suppression of "Mendelism-Morganism" in the Soviet Union and the Peoples' Republics under the political influence of the then recently deposed Soviet Lord of Biology, Trofim Lysenko. But this memorial Mass could have been thought to have had yet another symbolic meaning: a commencement exercise for the students of heredity, whose work actually began not a mere century ago, but ten thousand years earlier, in Neolithic times, and whose quest for the understanding of how like begets like was now about to reach its goal.

The capacity of living organisms to pass on their own qualities to their offspring is so obvious that it no doubt ranks as one of man's earliest scientific observations. Indeed, it was precisely the recognition of heredity and of the possibility of selective breeding that enabled the Stone Age denizens of the Near East to develop some of our domestic animals and crop plants from wild prototypes. This first success in biotechnology brought about the dawn of civilization—the transition from nomadic, food-gathering societies to sedentary, agricultural-urban societies in the Fertile Crescent in about 8000 B.C. The practical know-how gathered in millenia of breeding experience was passed on as magical or religious canon. For example, the Biblical stricture "Thou shalt not let thy cattle gender with a diverse kind; thou shalt not sow thy field with mingled seed" indicates that the ancient Hebrews were aware of the importance of maintaining pure lines of animals and plants. By classical times, breeding rules were being applied also to the human stock, as is exemplified by the infanticide of defective offspring practiced in the Greek city-states. These ancient rules and prescriptions for selection and breeding of stock were not significantly improved until the nineteenth century.

The philosophers of classical Greece gave some thought to hereditary processes. In the fifth century B.C., Hippocrates developed, or at least taught in his medical school, the first known theory of heredity. This theory held that a child possesses the qualities of its parent because the semen concentrates within it small representative elements from all parts—healthy and diseased—of the parental body. The corresponding parts of the filial embryo were then thought to be built up from the parental elements supplied by the semen. In accord with this view, Hippocrates believed in the hereditary transmission of acquired characters. For instance, he thought that the trait of long-headedness arose through the archaic social custom of artificially distorting the normal globular soft skull of the newborn infant. Aristotle, less than a century later, showed the inadequacy of the Hippocratic view. Aristotle argued that the filial embryo cannot have been reconstituted from representative elements collected from the parental bodies because:

1. Parents (and also plants) produce offspring endowed with parental traits (for instance, gray hair) that are manifest only in the post-reproductive stage of life.
2. The body is only the wrapping of the embryo, and hence the Hippocratic theory leads to the absurd inference that parental clothes and shoes also send their representatives to the semen.
3. Children of crippled and mutilated parents do not always show the defects of their progenitors.

Aristotle proposed, therefore, that rather than supplying the constituent elements of the embryo, the semen of the father provides the *plans* according to which the unformed blood of the mother is to be shaped into the offspring.

Thus Aristotle recognized that biological inheritance is not the passage through the generations of body part samples, but instead is attributable to the transmission of *information* for the embryonic development of the individual. This deep insight into the essence of heredity provided by Aristotle was forgotten for the next twenty-three centuries. What was remembered of Aristotelian reproductive biology consisted mainly of the description of fantastic hybrid matings between wildly different animal species. For instance, it was believed that a cross between a camel and a leopard had spawned the giraffe, and that eels come ashore to mate with snakes. The Renaissance, which had initiated the reawakening of interest in the physical sciences and the rejection of dogmatic superstition, produced few new insights into heredity. Indeed, it saw the rise of a notion even less sophisticated than the Hippocratic doctrine, namely the *preformation* theory, which envisaged the process of individual development as merely the unfolding of a preformed tiny midget, or *homunculus*, present in the father's semen, or in the mother's blood. Hence this view necessarily led to the belief that all later generations of the human race had already been preformed, one into the other, in—depending on the relative roles assigned to male and female in this infinite recessional system of Chinese boxes—Adam or Eve. Not until Mendel provided his radically novel insights did the new era dawn in which the mechanisms governing the self-reproduction of man and his fellow creatures became ultimately revealed.

For his "Experiments on Plant Hybrids" Mendel cultivated the common garden pea, *Pisum sativum* (Figure 1-2), in the garden of his Brno monastery. Mendel chose the pea plant for breeding experiments because its flower is so constructed as to render it naturally self-fertilizing: the pollen from another flower cannot gain access to the stigma, and hence the ovules of the flower are fertilized only by its own pollen. Nevertheless, it is possible to cross-fertilize a pea plant experimentally by opening the immature flower, removing its anther, which would bear the pollen, and later touching the stigma with pollen from another plant. Thus Mendel realized the possibility of breeding plants of exactly controlled descent. He had at his disposal various strains of *P. sativum* that differed from each other in a few, well-defined characters, such as seed color, seed morphology, or stem size. Each of these strains bred true, in that upon self-fertilization every progeny plant manifested the parental character. In his paper, Mendel reported as "Experiment 1" the following cross between two such strains. The ovules of flowers of a strain producing ordinary round seeds were cross-fertilized with pollen from flowers of a strain producing unusual, wrinkled seeds, and ovules of plants that produced wrinkled seeds were cross-fertilized with pollen from plants that produced round seeds. Several hundred *first-filial-generation hybrid* seeds resulted from this cross, *all of which were round.* In the next year, Mendel planted 253 of these round hybrid seeds, allowed the resulting pea plants to self-fertilize, and obtained 7324 *second-filial-generation* seeds from them. He found that of these seeds 5474 were round and 1850 wrinkled, giving a ratio of round : wrinkled = 2.96 : 1 (Figure

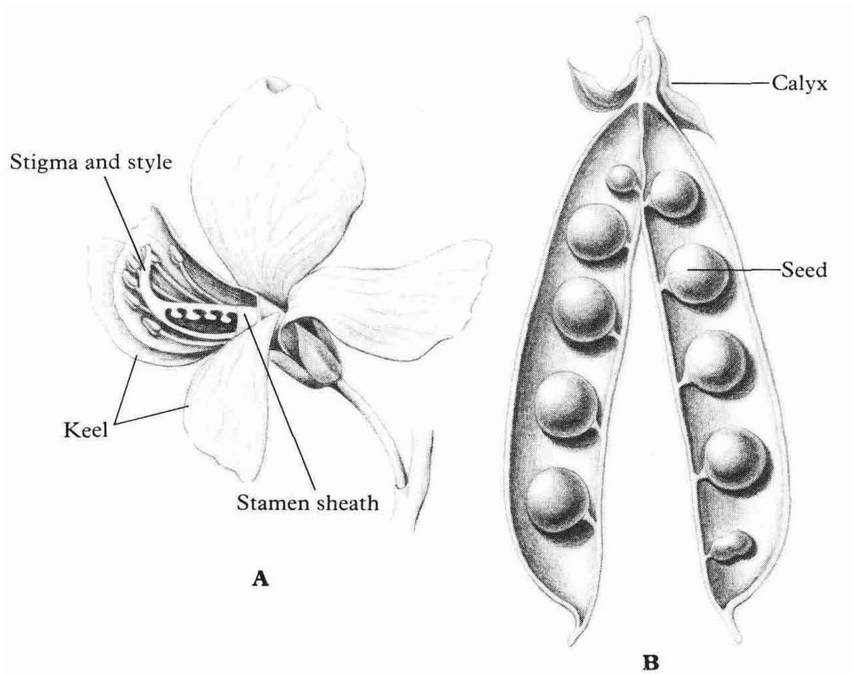


FIGURE 1-2
A The self-fertilizing flower of the garden pea. A portion of the keel, which encloses the reproductive organs, has been cut away to show the stamen, with its anthers bearing the male germ cells, or pollen, and the stigma, which receives the pollen from the anthers. The pollen grains then move along the style toward the ovary, where they fertilize the ovules, or female germ cells.
B The mature fruit (pod), containing the seeds that develop from the fertilized pea flower. [After J. B. Hill, H. W. Popp, and Alvin R. Grove, Jr., *Botany*, McGraw-Hill, New York, 1967.]

1-3). Six more analogous *monohybrid* crosses between plants having other single character differences all gave the same general result:

1. Of the two alternative parental characters, only one appears in the first filial generation.
2. The character that vanishes in the first filial generation reappears among one-fourth of the members of the second filial generation.

From these observations Mendel made a brilliant deduction—one that must be placed among the most astute intellectual contributions to our understanding of nature. He deduced that the hereditary characters of the pea are carried and passed on to the progeny as *discrete units*. Each pea must possess a *homologous pair* of such units, of which it has received one from the pollen and one from the ovum whose union gave rise to the seed whence it sprung. Of the two homologous units that produce such alternative characters as round and

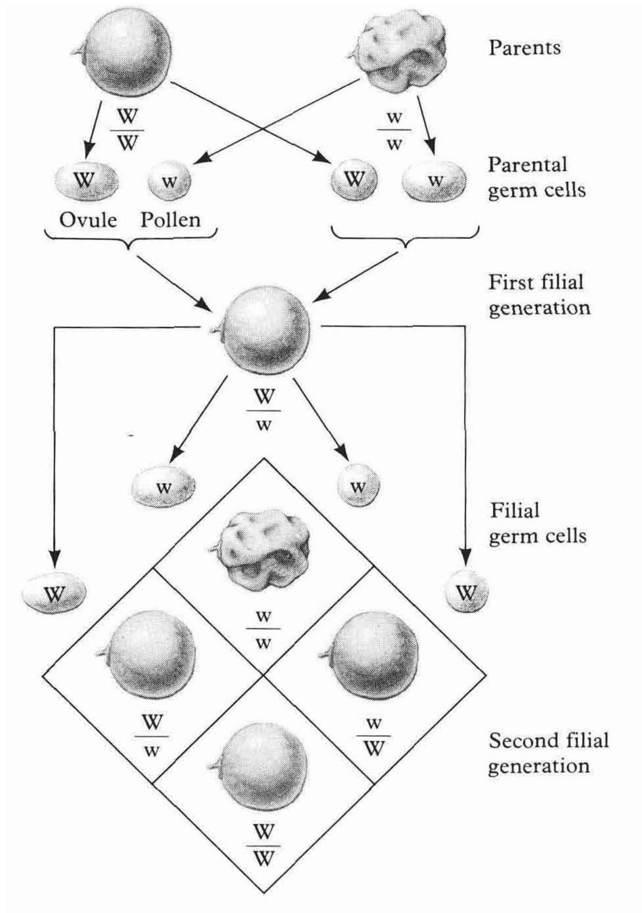
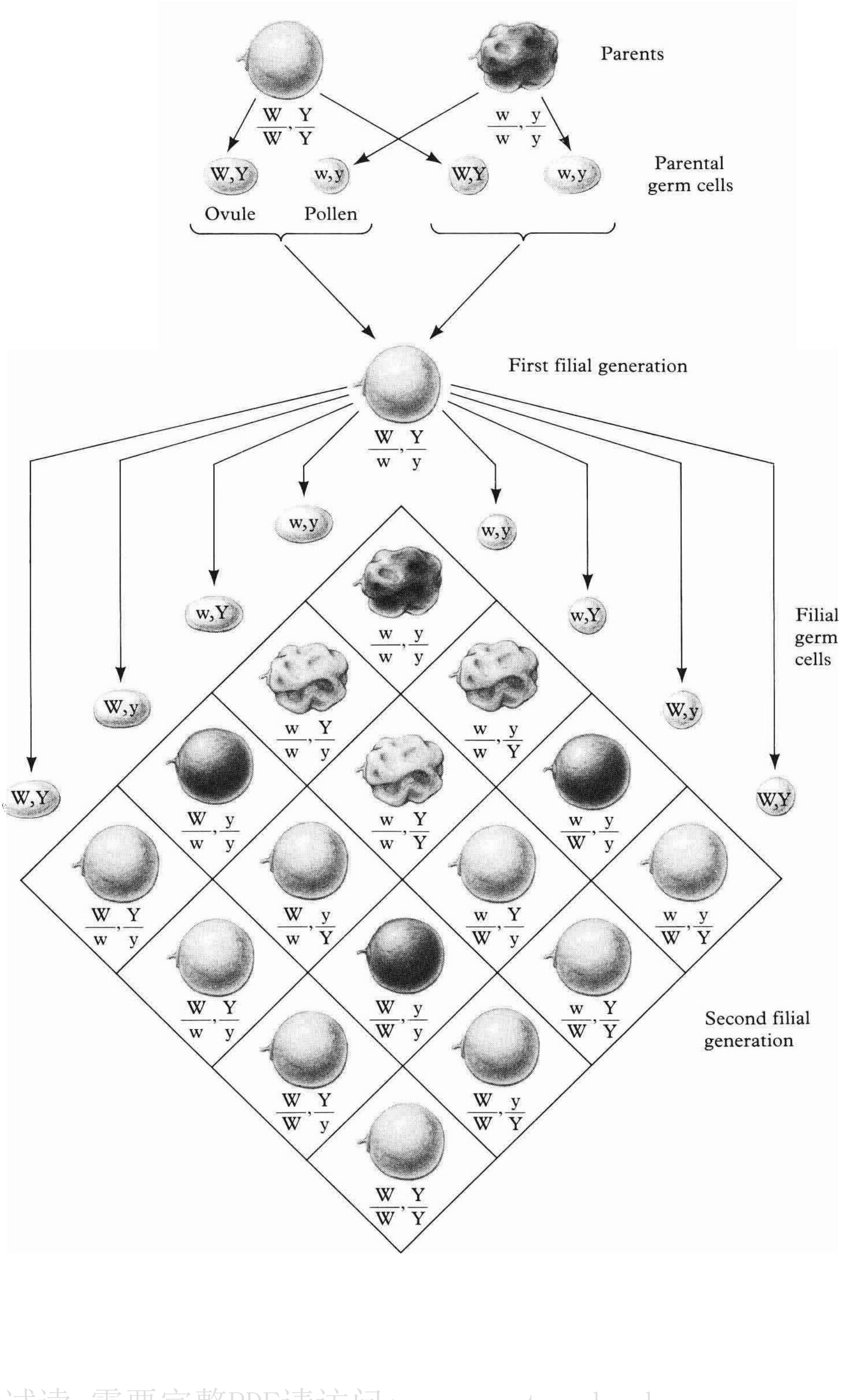


FIGURE 1-3
Mendel's monohybrid cross. The ovules of flowers of a pure strain of the garden pea producing ordinary round seeds were cross-fertilized with pollen from flowers of a pure strain producing unusual wrinkled seeds. The round-seed plant owes its character to the possession of a pair of *dominant* hereditary units, labeled W/W, and the wrinkled-seed plant to a pair of *recessive* units, labeled w/w. Plants of the first filial generation are all of the hybrid type W/w and hence show the dominant round-seed character. The flowers on plants grown from hybrid seeds of the first filial generation were allowed to self-fertilize. Since each such flower produces two types of ovules and two types of pollen, there will arise four types of seeds in the second filial generation: w/w, W/w, w/W, and W/W. Of these, only the w/w seeds show the recessive wrinkled character, whereas the other three types show the dominant round character.

wrinkled seed, one is *dominant* and the other is *recessive.* Hence in the first filial generation of hybrids only the character of the dominant unit (round) is manifest, even though the cryptic recessive unit (wrinkled) is also present in every plant. Upon self-fertilization of flowers of the first filial generation, however, four kinds of seeds will arise in equal frequency (Figure 1-3), of which only one has drawn a pair of recessive units. This causes the character of the recessive unit (wrinkled) to be manifest in only one-third as many members of the second filial generation as the character of the dominant unit (round).

Mendel's paper also reported the results of *dihybrid* crosses between a pair of pea strains that differed from each other in *two* alternative characters. The seeds of one strain were yellow and round, and the seeds of the other were green and wrinkled. The seeds of the first filial generation of this cross were all yellow-round, a result that was in agreement with the inference he had previously made on the basis of crosses between seeds differing in only one character —that yellow and round are dominant hereditary units *vis-à-vis* their recessive


Parents
W/W, Y/Y
w/w, y/y
Parental germ cells
W,Y
w,y
W,Y
w,y
Ovule
Pollen
First filial generation
W/w, Y/y
Filial germ cells
W,Y
W,y
w,Y
w,y
w,y
w,Y
W,y
W,Y
Second filial generation
w/w, y/y
w/w, Y/y
w/w, y/Y
W/w, y/y
w/w, Y/Y
w/W, y/y
W/w, Y/y
W/w, y/Y
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