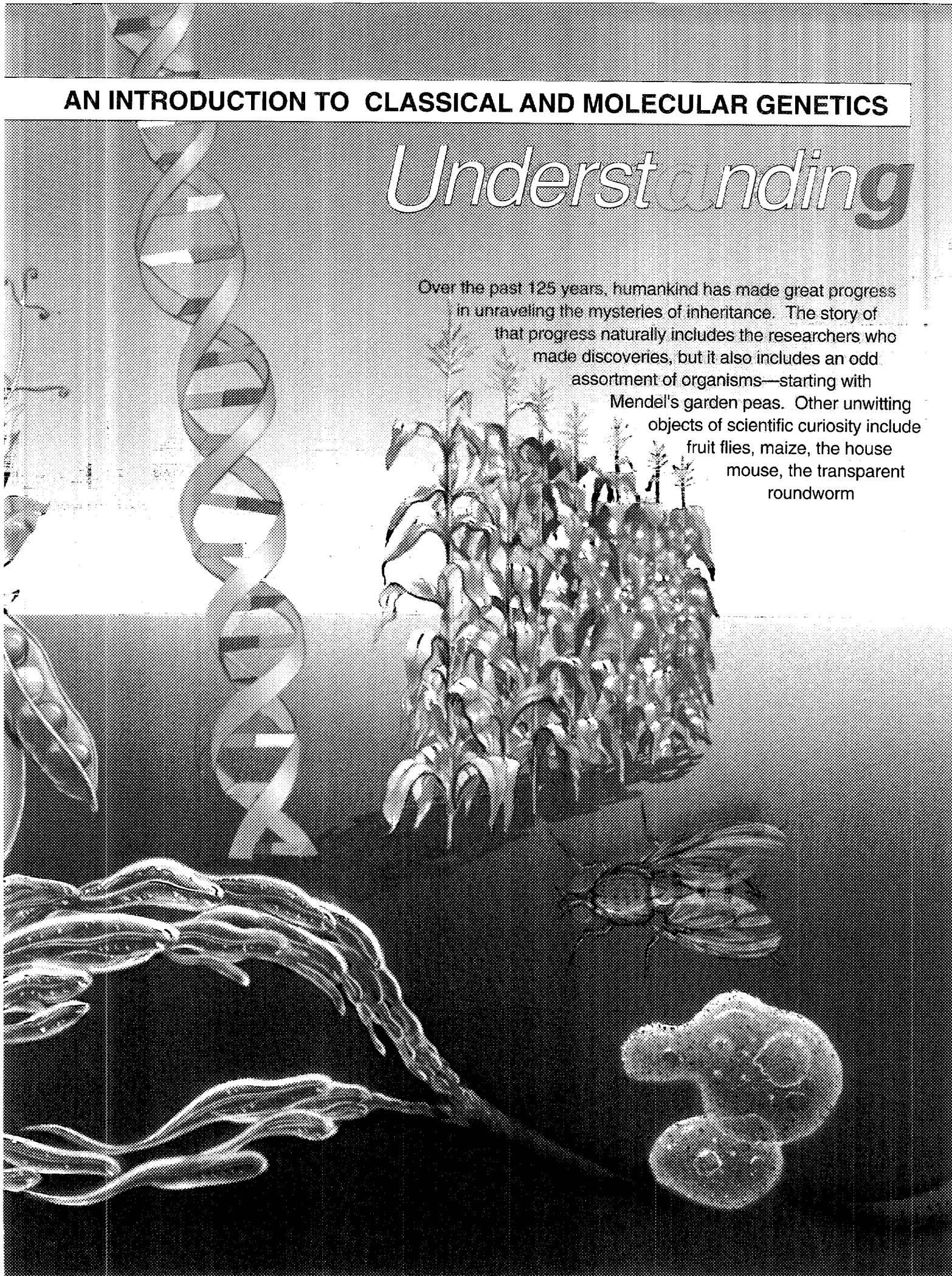
AN INTRODUCTION TO CLASSICAL AND MOLECULAR GENETICS

Understanding

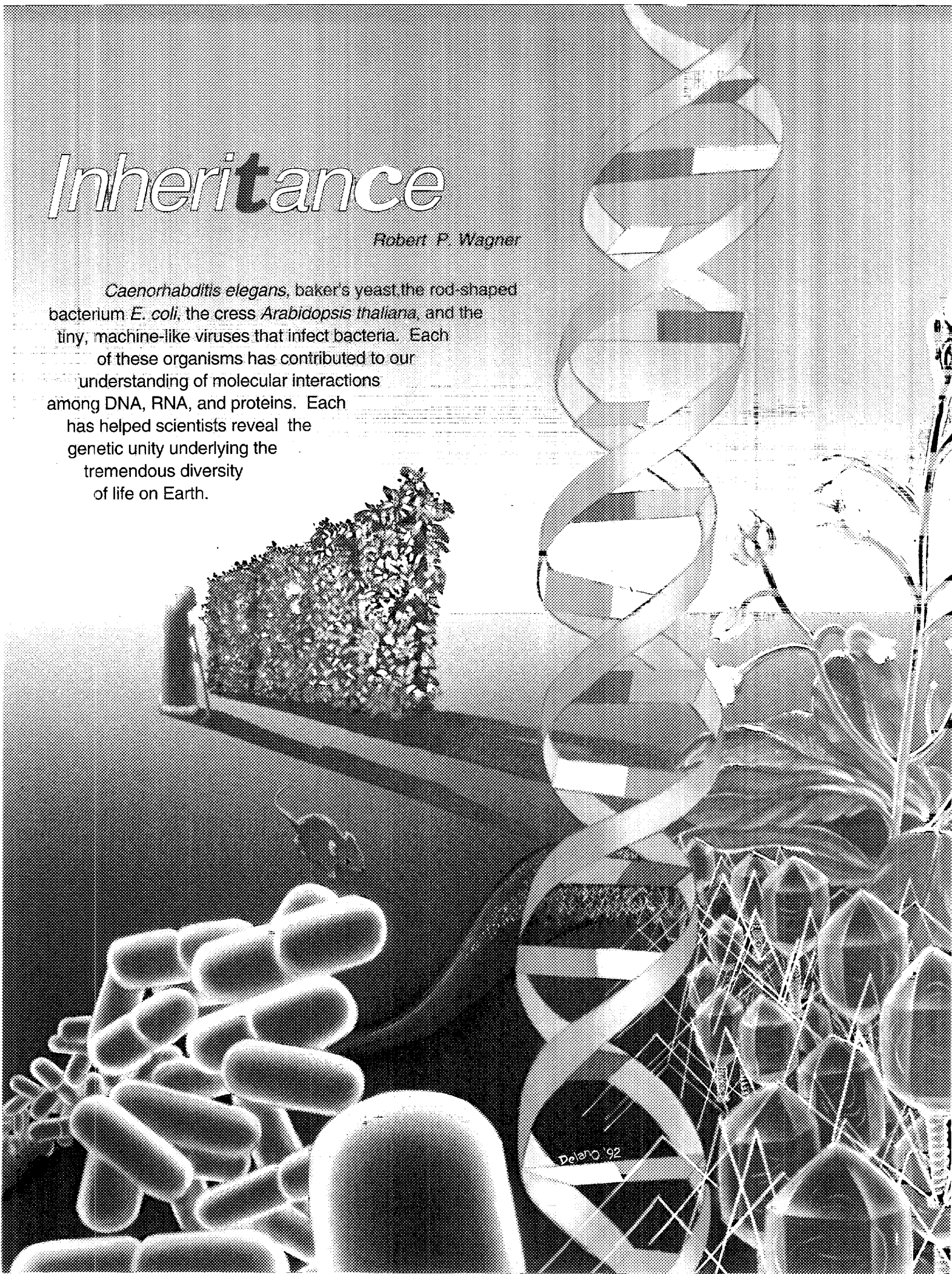


Over the past 125 years, humankind has made great progress in unraveling the mysteries of inheritance. The story of that progress naturally includes the researchers who made discoveries, but it also includes an odd assortment of organisms—starting with Mendel's garden peas. Other unwitting objects of scientific curiosity include fruit flies, maize, the house mouse, the transparent roundworm

Inheritance

Robert P. Wagner

Caenorhabditis elegans, baker's yeast the rod-shaped bacterium *E. coli*, the cress *Arabidopsis thaliana*, and the tiny, machine-like viruses that infect bacteria. Each of these organisms has contributed to our understanding of molecular interactions among DNA, RNA, and proteins. Each has helped scientists reveal the genetic unity underlying the tremendous diversity of life on Earth.



That like begets like—that what is now called a species begets offspring of the same species—must have been evident to the earliest humans. Recognition of the inheritance of variations within a species must also have come early, since domestication of animals undoubtedly involved elimination of individuals with undesirable characteristics (a penchant for human flesh, for example). The first animals to be domesticated may well have been members of the dog family, which were used as food, and domestication of canines may have started even before the advent of *Homo sapiens*. The remains of an old hominid relative of ours, *Homo erectus* (also known as Java or Peking man), have been found associated with those of a dog-like animal in 500,000-year-old fossils. The earliest canine remains associated with our own species are a mere 12,000 years old. The domestication of food plants probably began between 8000 and 9000 years ago, although some authorities contend that the domestication of cereals preceded that of most animals.

Humans must also have very early related mating between "male" and "female" animals, including humans, with the subsequent issuance of offspring. Sexual reproduction in plants was probably recognized much later—many plants, after all, are discreetly bisexual—but at least 4000 years ago, as evidenced by the Babylonians' selective breeding, through controlled pollination, of the date palm (*Phoenix dactylifera*), which occurs as separate male and female trees. (The dates borne by a female tree result from fertilization of its eggs by sperm-containing pollen from male trees.)

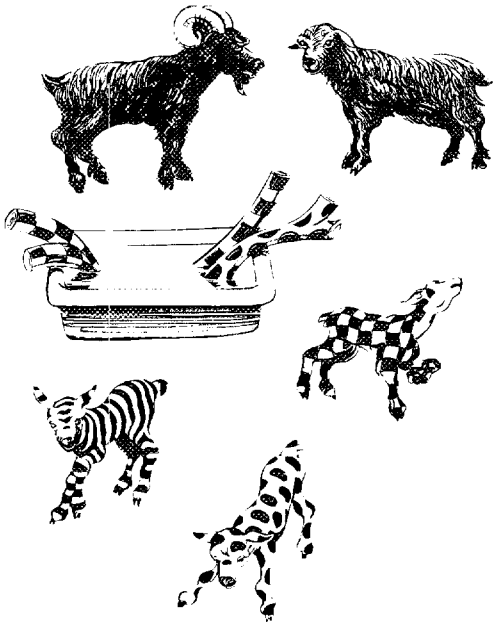
The oldest recorded thoughts about heredity appear in the religious writings of the ancient Hindus and Jews, which reveal recognition of the heritability of disease, health, and mental and physical characteristics. The caste system of the Hindus, the hereditary priesthood among the Jews of the tribe of Levi, and later, in Homer's time, the inheritance of the gift of prophecy are a few reflections of ancient thinking about the link between successive generations of humans. Some of those ideas, which of necessity were based primarily on philosophical outlook rather than scientific fact, are discussed briefly in "Early Ideas about Heredity."

The Dawn

The first significant advances toward our current understanding of inheritance came in the late Renaissance with the work of the English physician William Harvey (1578–1657) and the invention of the microscope (circa 1600). Harvey is best known for his discovery of the dynamics of the circulation of the blood, but he also propounded a new view about the relative importance of the contributions of male and female animals to the creation of offspring. Previously, the female contribution, the egg, had been regarded as mere matter, matter that assumes a form dictated entirely by the male's semen. But Harvey proposed that both egg and semen guide the development of an offspring. His observation of the eggs of many species led him to conclude (in *De generatione animalium*, 1651) that "*ex ovo omnia*." That everything arises from an egg was meant to apply to humans also, even though Harvey had never seen the eggs of humans or any other live-bearing creature.

EARLY IDEAS ABOUT HEREDITY

Ancient beliefs about heredity included the idea that inborn characteristics are inherited from parents, as well as the idea that they could be affected by external influences on the parents at conception or during pregnancy. The biblical story of Jacob's wages (Genesis, chapter 30) combines both. Jacob had agreed to tend the flock of his uncle and father-in-law, Laban, if he could take when he left all the unusually colored animals: the sheep with dark wool and the goats with white streaks or speckles. But Laban, a deceitful and greedy man, took his few such animals three days' journey away. The remaining stock he assumed would not produce offspring of the colorations Jacob had named. However, Jacob peeled tree



branches to make them striped and spotted and stood them in the watering troughs when the stronger goats were mating nearby. The kids from those matings, unlike their parents, had the markings that made them his, and they were more vigorous than the offspring of the weaker goats. He herded the sheep so they faced Laban's dark-colored goats; they then bore dark-colored lambs. Today the appearance in offspring of characteristics different from those of either parent can be attributed to the combined effects of the genetic contributions of each parent (see "Mendelian Genetics").

The ancient Greeks gave considerable attention to human inheritance in their writings. Plato, for example, made cogent statements about human traits being determined by both parents. He emphasized that people are not completely equal in physical and mental characteristics and that each person inherits a nature suited to fulfilling only certain societal functions. Also prominent in the thinking of the early Greeks was the inheritance of acquired characteristics. Aristotle, for example, wrote that

> children are born resembling their parents in their whole body and their individual parts. Moreover this resemblance is true not only of inherited but also of acquired characters. For it has happened that the children of parents who bore scars are also scarred in just the same way in just the same place. In Chalcedon, for example, a man who had been branded on the arm had a child who showed the same brand letter, though it was not so distinctly marked and had become blurred.

The idea that external influences play a role in heredity persisted even until the early part of the twentieth century. We now know that the idea contains some truth. For example, ionizing radiation, many chemicals, and infection by some viruses can cause heritable changes, or mutations, but generally those changes are entirely random and cannot be directed toward specific outcomes.

One of the more remarkable theories about human inheritance, pangenesis, was developed in about the fifth century B.C. and espoused by Hippocrates and his followers. According to that theory, semen was formed in every part of the male body and traveled through the blood vessels to the testicles, which were merely repositories. Variations of the theory lasted well into the ninteenth century A.D. and were even accepted by Charles Darwin. Pangenesis was for some reason dominant in the thinking of the philosophers and theologians of the Middle Ages. Albertus Magnus (1193–1280), his pupil Thomas Aquinas (1225–1274), and the naturalist Roger Bacon (circa 1220–1294) all accepted pangenesis as a fact. One variant of the theory was the idea that both male and female produced semen. According to Paracelsus (1493–1541), semen was an extract of the human body containing all the human organs in an ideal form and was thus a physical link between successive generations.

Also prevalent during the Middle Ages was the concept of entelechy, the Aristotelian idea that the way an individual develops is determined by a vital, inner force. The determining force is provided by the male and transmitted in his semen. The female provides no semen but only, so to speak, raw material. Aristotle compared the roles of male and female in the creation of an offspring with the roles of sculptor and stone in the creation of a sculpture.

Other forms of vitalism continued to be popular even up to the beginning of the twentieth century primarily because people lacked knowledge about the nature of the physical connection between generations of animals and plants.

With his naked eye Harvey could see no form in a newly laid, fertilized chicken egg. But he assumed the form that did appear later arose epigenetically from matter that has some sort of inherent, though invisible, organization. The theory of epigenesis—that an organism arises from structural elaboration of formless matter rather than by enlargement of a preformed entity—dates back to Aristotle, but Harvey differed from Aristotle in seriously doubting that the living can arise from the nonliving. Experimental justification for his doubt came about a century later.

Thoughts about heredity would probably not have advanced beyond Harvey's had it not been for the compound microscope, an invention credited sometimes to Zaccharias Janssen and sometimes to Galileo. Other Renaissance men noted for their discoveries with the microscope and improvements to its design are regarded as the founders of microscopy: Nehemiah Grew (1641–1712), Robert Hooke (1635–1703), Antoni van Leeuwenhoek (1632–1723), Marcello Malpighi (1628–1694), and Jan Swammerdam (1637–1680). Their observations—among which were sperms in semen and structural elements, dubbed cells by Hooke, in plant and animal tissues—formed the foundations of the science now called cell biology.

Users of the early, low-resolution microscopes could (and did) let their imaginations run wild. Some thought they saw miniature humans, homunculi, preformed in human sperms; others saw tiny animals, animalcula, preformed in animal eggs. Those apparitions led to resurrection of the theory of preformation originally propounded by Democritus and other Greeks. In the eighteenth century the preformation theory developed into the encapsulation theory, which stated that, at the time of creation, all future generations were packaged, one inside the other, within the primordial egg or sperm. Logically, all life would come to an end when the last homunculus or animalculum was born. The encapsulation theory died—because it was ridiculous—although many eminent biologists were its fierce advocates up to the beginning of the nineteenth century.

The higher-resolution microscopes of the later half of the eighteenth century allowed Caspar Friedrich Wolff (1734–1794) to observe the development of chicken embryos. His work clearly showed that the components of a new organism are not preformed but, as stated two millenia before by Aristotle and a century before by Harvey, arise from the undifferentiated matter of the fertilized egg.

The Great Awakening

Modern biology may be said to have been born in the nineteenth century, several hundred years after the beginnings of modern chemistry and physics. Earlier biologists were either physicians or naturalists (what we now call botanists and zoologists), and their work focused on structure, physiology, and classification. But the nineteenth century brought several developments that were basic to emergence of the newer branches of biology, including cell biology and genetics.

The Rise of Cell Biology. During the first half of the nineteenth century, evidence accumulated for the so-called cell theory, which states that the cell is the structural and functional unit of all organisms. The diversity of cell shapes and sizes was noted (see "The Variety of Cells"), and various intracellular structures were observed (see "Components of Eukaryotic Cells"). Of particular importance to genetics is the membrane-bound intracellular structure called the nucleus, which was found to be a common feature of the cells of all organisms more complex than bacteria and blue-green algae. Organisms possessing a nucleus were classified as eukaryotes, and organisms lacking a nucleus were classified as prokaryotes.

Later, during the early 1850s, came the momentous finding, embraced in the aphorism *omnis cellula e cellula*, that cells divide to form new cells. A leading proponent of the idea that all cells come from cells was the German physician Rudolph Virchow (1821–1902). A cancer specialist, among other things, Virchow asserted that cancer cells arise from cells pre-existing in the body and do not, as earlier physicians had thought, arise by spontaneous generation from unorganized matter.

Another development was the realization that gametes (sperms and eggs) are also cells, in particular cells specialized for transmitting information from one generation of a sexually reproducing organism to the next. The remarkable difference in size between sperms and eggs was found to be due to cell components other than their nuclei, and that observation, coupled with the belief that sperms and eggs contain the same amount of hereditary information, indicated that hereditary information resides in the nuclei of gametes. The nucleus was found to be the site also of the information transmitted from one cellular generation to the next.

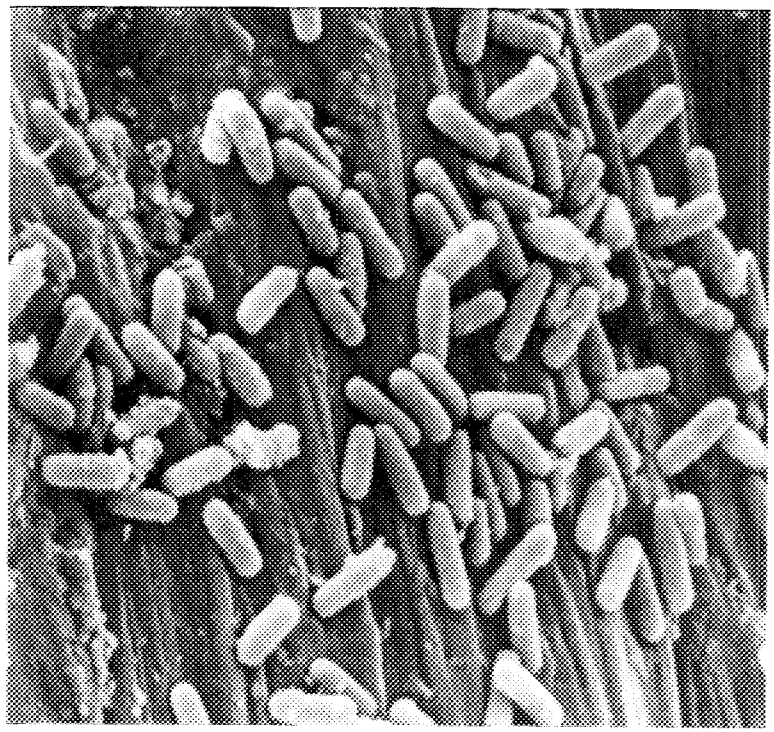
The above developments led to formulation of the law of genetic continuity, which succinctly summarizes what was probably the most important advance toward the understanding of living systems up to that time: Life comes only from life through the medium of cells.

By the late 1880s hereditary information had been localized further to intranuclear elements that can be seen with the microscope during the mitotic phase of the cell cycle, the phase that culminates in cell division (see "The Eukaryotic Cell Cycle"). The elements, which were named chromosomes because they can be stained (selectively colored) with certain dyes, are most easily observed during the portion of the mitotic phase called metaphase. (We now know that each "metaphase chromosome" consists of two duplicates of a single chromosome bound together along a more or less central region.)

Facts accumulated about chromosomes (see "Chromosomes: The Sites of Hereditary Information"). All the somatic cells (cells other than gametes) of a sexually reproducing organism have the same even number of chromosomes, the so-called diploid number, whereas all its gametes have the same so-called haploid number of chromosomes, which is exactly one-half the diploid number. Furthermore, the diploid and

THE VARIETY OF CELLS

Cells vary in shape from the most simple to the indescribably complex. Shown here are electron micrographs of a few examples from nature's cornucopia.



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***Escherichia coli*, the most studied of all bacteria**

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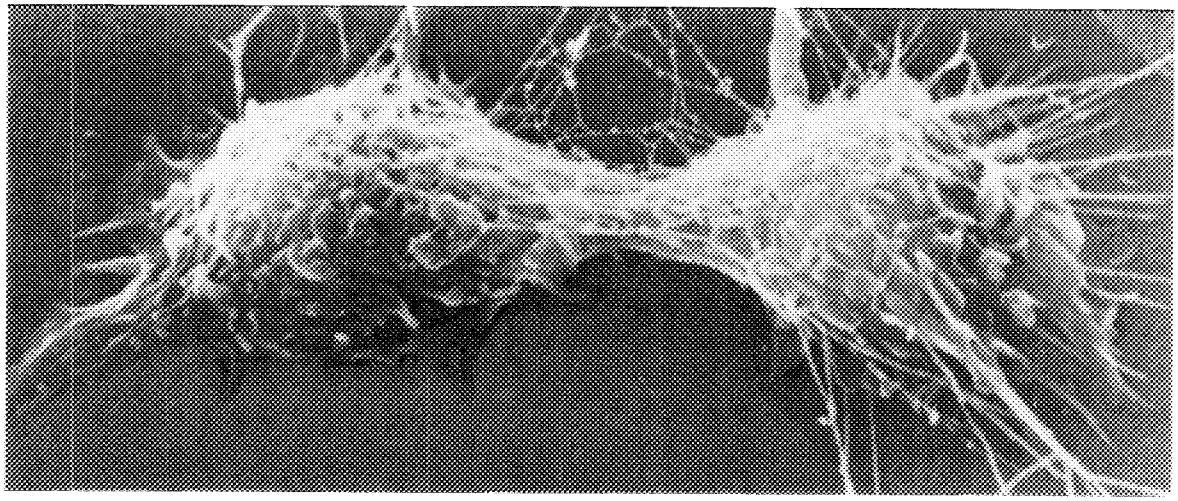

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Mouse fibroblast during the final stage of cell division

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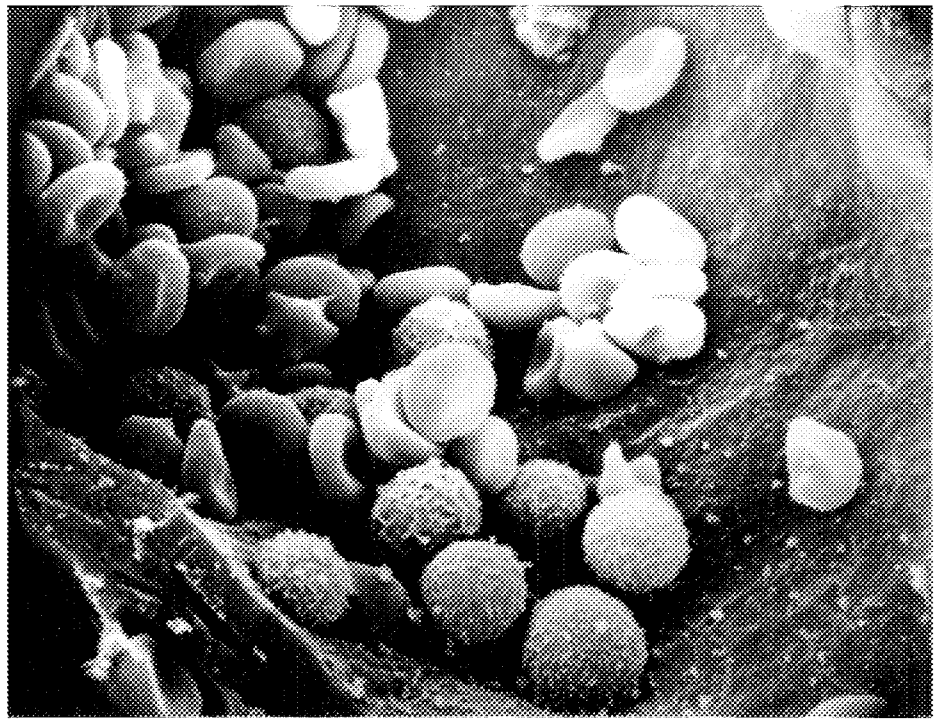

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Human red blood cells (biconcave) and white blood cells (rounded)

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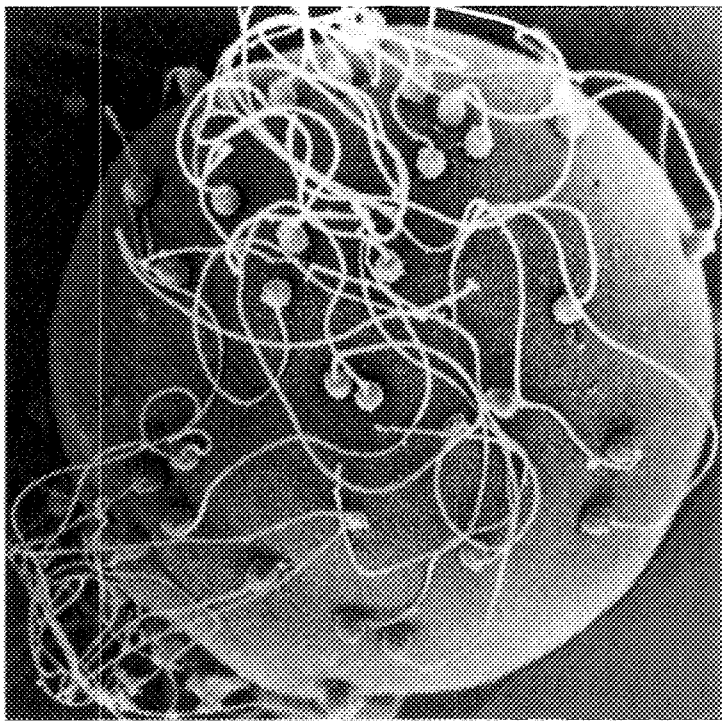

A clam egg with many sperms bound to its surface

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COMPONENTS OF EUKARYOTIC CELLS

Eukaryotic cells, unlike prokaryotic cells, possess membrane-bound internal structures called organelles. The organelles common to eukaryotic plant and animal cells include mitochondria (the sites of energy production by oxidation of nutrients), a Golgi apparatus (where various macromolecules are modified, sorted, and packaged for secretion from the cell or for distribution to other organelles), an endoplasmic reticulum (the principal site of protein synthesis), and a nucleus (the residence of chromosomes and the site of DNA replication and transcription). The nucleolus is the site of ribosomal-RNA synthesis. The organelles unique to plant cells are chloroplasts (the sites of photosynthesis in green plants) and vacuoles (water-filled compartments that serve as space fillers and as storage vessels). Plant cells differ from animal cells also in being surrounded by a cellulose cell wall, a much more rigid form of the extracellular matrix that surrounds animal cells.

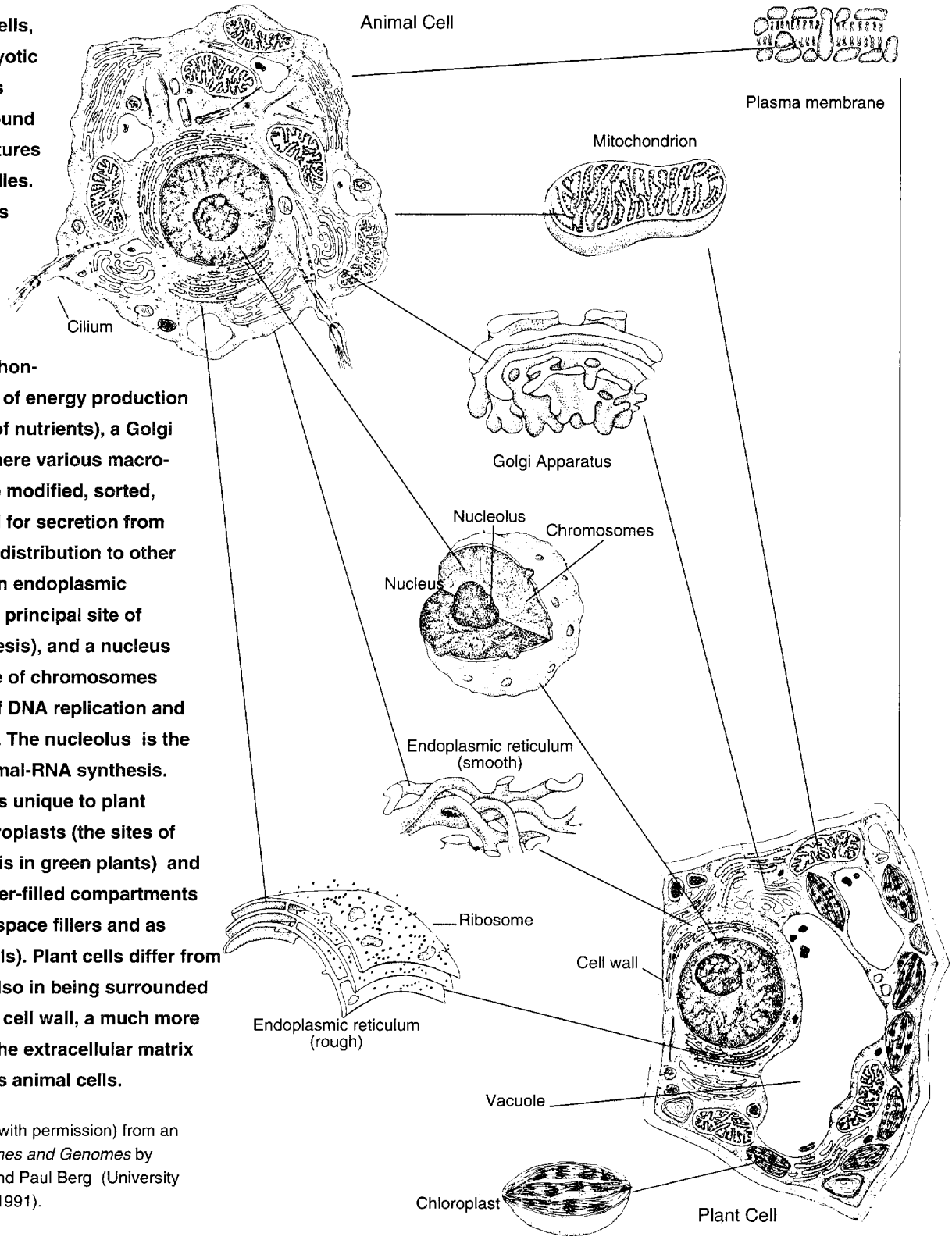
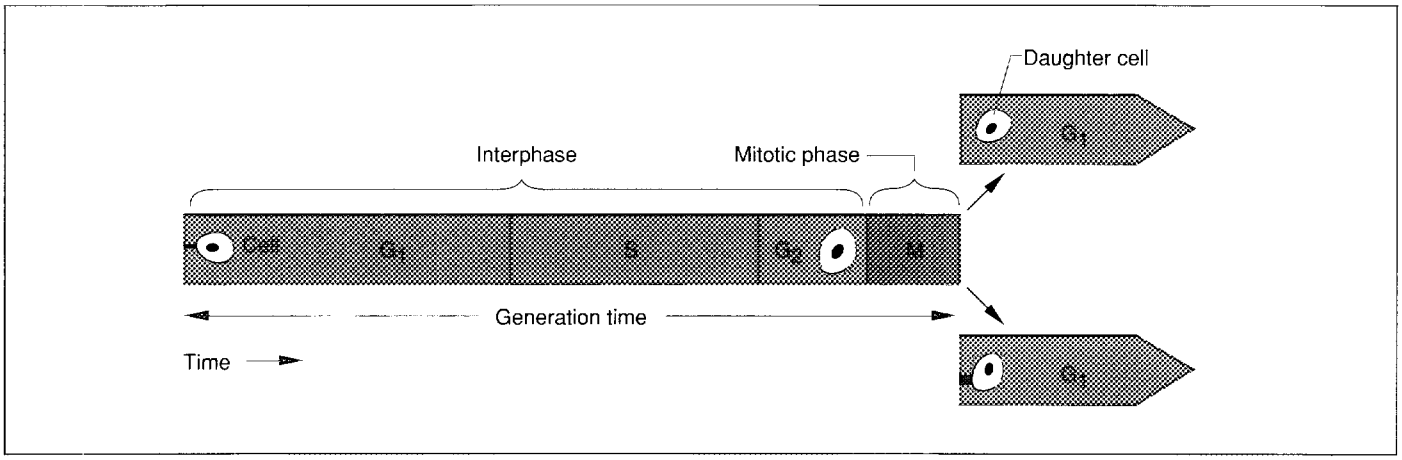


Figure adapted (with permission) from an illustration in *Genes and Genomes* by Maxine Singer and Paul Berg (University Science Books, 1991).

THE EUKARYOTIC CELL CYCLE



The term "cell cycle" refers collectively to the events that occur within a eukaryotic cell between its birth by mitosis and its division, again by mitosis, into two daughter cells. The cell may be either a one-celled organism such as baker's yeast (*Saccharomyces cerevisiae*) or a somatic cell of a multicellular organism. Early studies of the eukaryotic cell cycle concentrated on the microscopically visible and dramatic physical events of the cell-division, or mitotic, phase (M). Onset of the mitotic phase is signaled by the appearance of microscopically visible worm-like bodies within the nucleus, that is, by the condensation of duplicated chromosomes into a much less diffuse configuration. The mitotic phase ends when the cell separates into two daughter cellls, each of which then embarks on its own cycle. (Details of the mitotic phase are presented in "Mitosis.")

Because the early microscopic studies revealed little physical activity during the portion of the cell cycle that precedes the mitotic phase (other than a relatively small increase in cell size), that portion was inappropriately named the resting phase, or interphase. We now know that most of the biosynthetic activity required of a cell—both for its own maintenance and reproduction and for its function or functions as a constituent of a multicellular organism—occurs during interphase.

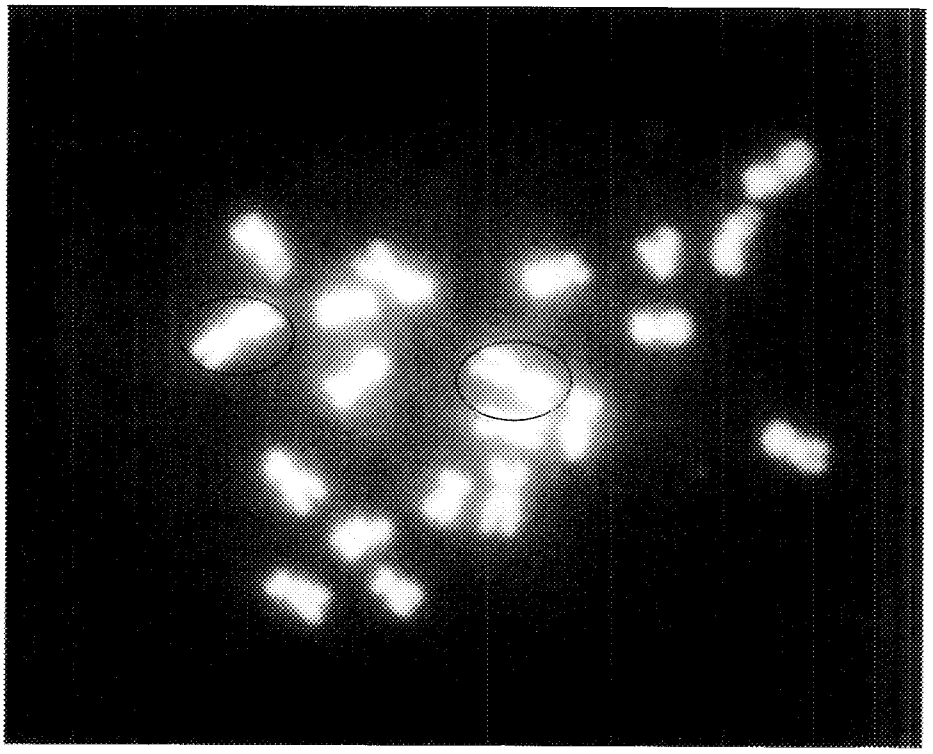
Most of the biochemicals produced by a cell are synthesized throughout interphase. DNA is a notable and easily detected exception, and for that reason interphase is subdivided into the period between cell birth and the onset of DNA synthesis (G_1), the period of DNA synthesis (S), which ends when all the nuclear DNA has been replicated and hence the number of chromosomes has doubled, and the period between the end of DNA synthesis and the beginning of the mitotic phase (G_2). After a cell has entered S, it is committed to completing the cell cycle, even when environmental conditions are extremely adverse.

The length of the cell cycle, the generation time, varies with environmental conditions and among species and cell types. For example, epithelial cells, the cells that line the interior and exterior surfaces of the human body, have relatively short generation times (about eight hours); fibroblasts, cells that assist in healing wounds, complete their cell cycle only on demand; mature red blood cells never undergo mitosis; and embryonic cells divide very rapidly. Observed generation times for those cells that do have a regular cycle range from about a few minutes to a few months. The variation in generation time is due mainly to a variation in the length of G_1 and of G_2. The mitotic phase of most species and most cell types occupies only about 10 percent of the generation time.

The cell cycle of bacteria, in addition to being shorter (typically less than an hour), is also less complex. In particular, DNA is synthesized continuously, the two copies of the single bacterial chromosome do not undergo extensive condensation before cell division, and a mechanism simpler than the one illustrated in "Mitosis" assures parceling out of one chromosome copy to each daughter cell.

CHROMOSOMES: the sites of hereditary information

Within the nucleus of each cell of a eukaryotic organism are a number of chromosomes, each composed of a single molecule of DNA (see "DNA: Its Structure and Components") and a roughly equal mass of proteins (primarily the proteins called histones). The DNA molecule carries hereditary information; the proteins help effect the ordered condensation, or compaction, of the very long, very thin DNA molecule. During most of a cell's life, its chromosomes are too decondensed to be visible with an optical microscope. However, during metaphase, a phase preparatory to cell division (see "Mitosis" and "Meiosis"), the chromosomes become highly condensed and hence easily visible. Most studies of chromosomes are therefore carried out on chromosomes extracted from cells arrested at metaphase. Each such "metaphase chromosome" consists in reality of two duplicates of a single chromosome bound together along a somewhat constricted region called a centromere. The three micrographs of metaphase chromosomes shown here illustrate some general facts about chromosomes.



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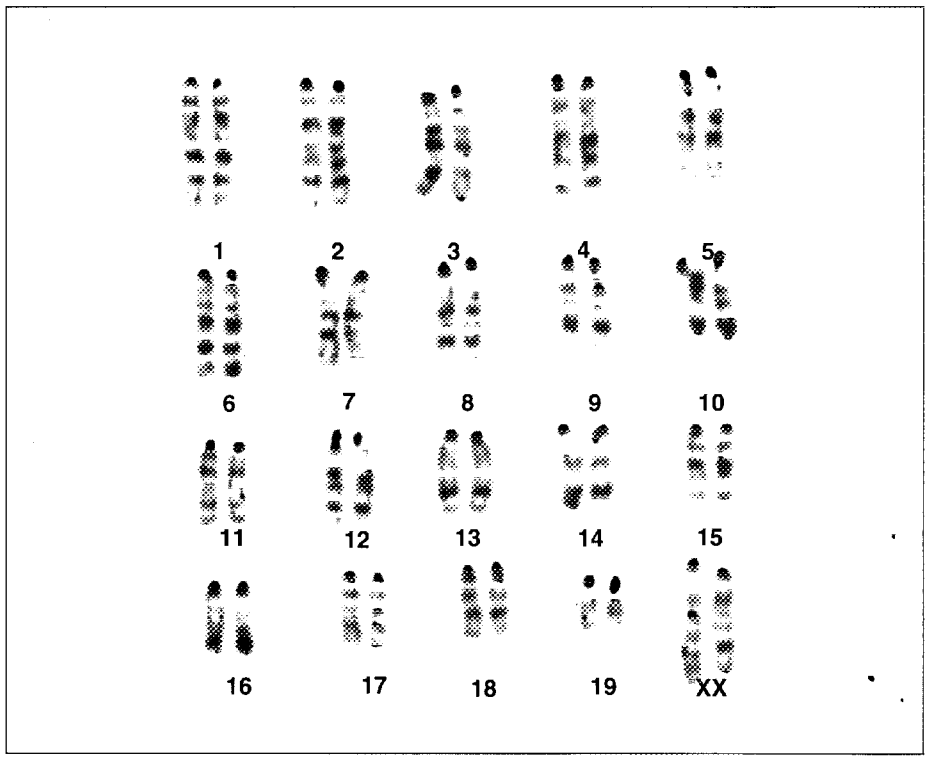
Shown above are the metaphase chromosomes extracted from a root-tip cell of maize (*Zea mays*). The chromosomes were stained with a fluorescent dye and photographed through an optical microscope while being illuminated by a laser that excites the dye's fluorescence. (The chromosomes could have been stained instead with a nonfluorescent dye.) A total of twenty metaphase chromosomes is visible in the micrograph, and any somatic cell (any cell other than an egg or a sperm) of any *Zea mays* plant possesses that same number of metaphase chromosomes. In general, all the somatic cells of all the members of a species possess the same even number of metaphase chromosomes, called the diploid chromosome number. The diploid chromosome number varies erratically from species to species: the known values range from 2 to many hundreds. (Note that the diploid chromosome number is not a measure of a species' evolutionary status.) The twenty metaphase chromosomes of *Zea mays* obviously exhibit different morphologies, that is, different sizes and centromere positions. However, even the untrained observer might notice that the two highlighted metaphase chromosomes look very much alike. In fact, the twenty metaphase chromosomes of *Zea mays* can be grouped into ten homologous, or morphologically indistinguishable, pairs. The metaphase chromosomes of all eukaryotic species occur as homologous pairs, and that general fact is due to the occurrence of chromosomes themselves as homologous pairs. Furthermore, the homology of a pair of chromosomes is due to a high degree of similarity between the base sequences of their constituent DNA molecules. (Micrograph courtesy of Paul Jackson and Jérôme Conia.)

Shown at right are the metaphase chromosomes extracted from a somatic cell of a house mouse (*Mus musculus*). To help identify homologous pairs, the chromosomes were stained with a dye called Giemsa that produces a pattern of dark and light bands, a pattern that varies from one homologous pair to another. The chromosome images have been grouped in homologous pairs and arranged in order of decreasing size. Such a display of metaphase chromosomes is called a karyotype. The last entry in the karyotype is the pair of chromosomes that are involved in determining sex. Because this particular mouse cell posseses two *homologous* sex chromosomes, it is a cell from a female mouse. Cells of a male mouse possess two nonhomologous sex chromosomes, one X chromosome and a smaller Y chromosome.



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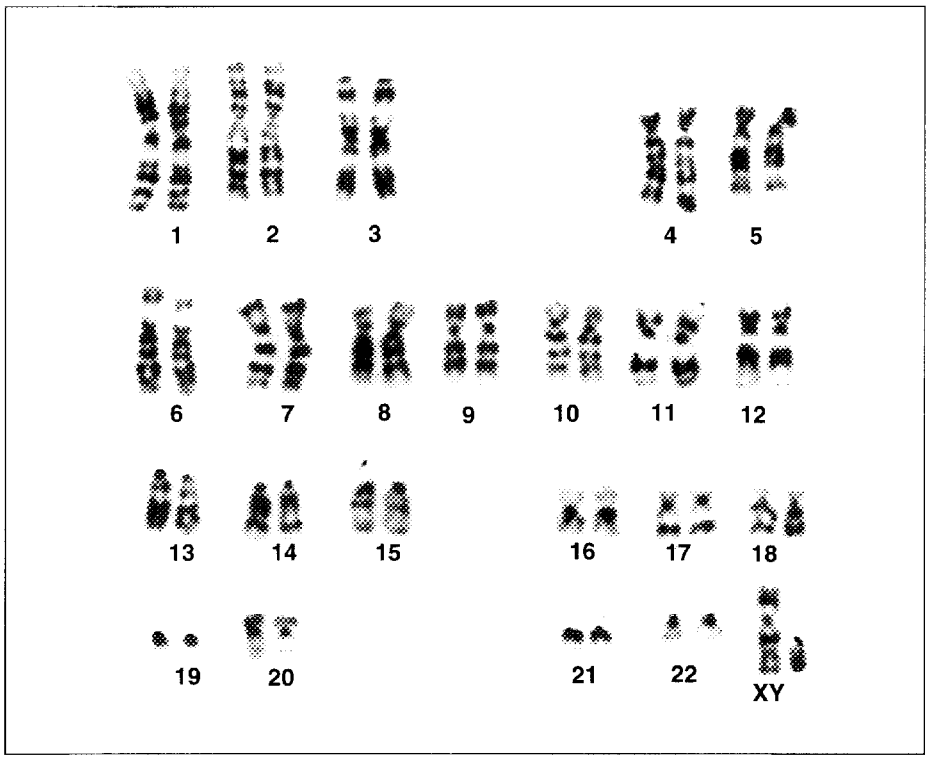


Shown at left is the karyotype of a human prepared from the Giemsa-stained metaphase chromosomes of a lymphocyte. Note the twenty-two homologous pairs of autosomes (chromosomes other than sex chromosomes) and the two nonhomologous sex chromosomes. The nonhomology of the sex chromosomes indicates that this is the karyotype of a male human, namely of the well-known cytogeneticist T. C. Hsu of the University of Texas System Cancer Center. (Both of the karyotypes on this page were provided by T. C. Hsu.)

haploid chromosome numbers are constant among different members of the same species but vary among different species. For example, all somatic cells of all members of the species *Homo sapiens* contain forty-six chromosomes, all somatic cells of all members of the species *Drosophila melanogaster* (a fruit fly) contain eight chromosomes, all somatic cells of all members of the species *Pisum sativum* (the garden pea) contain fourteen chromosomes, and all somatic cells of all members of the species *Mus musculus* (the house mouse) contain forty chromosomes. And all the gametes of all members of each of the above species contain twenty-three, four, seven, and twenty chromosomes, respectively. Second, the metaphase chromosomes within a single cell vary morphologically (in size and shape), but the variations remain constant among all cells of all members of a single species. (We now know that exceptions to the above generalizations occur and that the exceptions are often causes or symptoms of disease.)

The morphological differences among the metaphase chromosomes of a species led to recognition that metaphase chromosomes occur as morphologically indistinguishable (homologous) pairs. Although the members of a pair of homologous metaphase chromosomes are indistinguishable by any low-resolution physical technique, they do differ, as we now know, in fine details of the nucleotide sequences of their constituent DNA molecules. The occurrence of metaphase chromosomes as morphologically indistinguishable pairs is due to the occurrence of chromosomes themselves as homologous pairs, pairs whose constituent DNA molecules have nearly identical nucleotide sequences.

An exception to the occurrence of chromosomes as homologous pairs should be noted. Males of some species, including all mammals and *Drosophila melanogaster*, possess two chromosomes, called the X and Y chromosomes, that do not form a homologous pair, the Y chromosome generally being much smaller than the X chromosome. Females of such species possess two X chromosomes, each of which is homologous to the other and to the X chromosome of the male. Collectively, the X and Y chromosomes are called sex chromosomes; the remaining chromosomes are called autosomes. In the case of humans and other placental mammals, the presence of a Y chromosome is necessary for maleness (the presence of testes), but in the case of other species, including *D. melanogaster*, the presence of a Y chromosome, although necessary for fertility, is not necessary for maleness.

Also observed during the late nineteenth century were microscopic details of cell division and the effect of cell division on chromosomes. Mitosis, the type of cell division undergone by all somatic cells other than the immediate precursors of gametes, was found to yield two daughter somatic cells with the same diploid number of chromosomes as the mother cell (see "Mitosis"). Furthermore, the German zoologist Theodor Heinrich Boveri (1862–1915) found that the metaphase chromosomes of a mother cell and a daughter cell had the same morphologies. Those observations indicated that each chromosome in the mother cell is somehow duplicated before the cell undergoes mitosis.

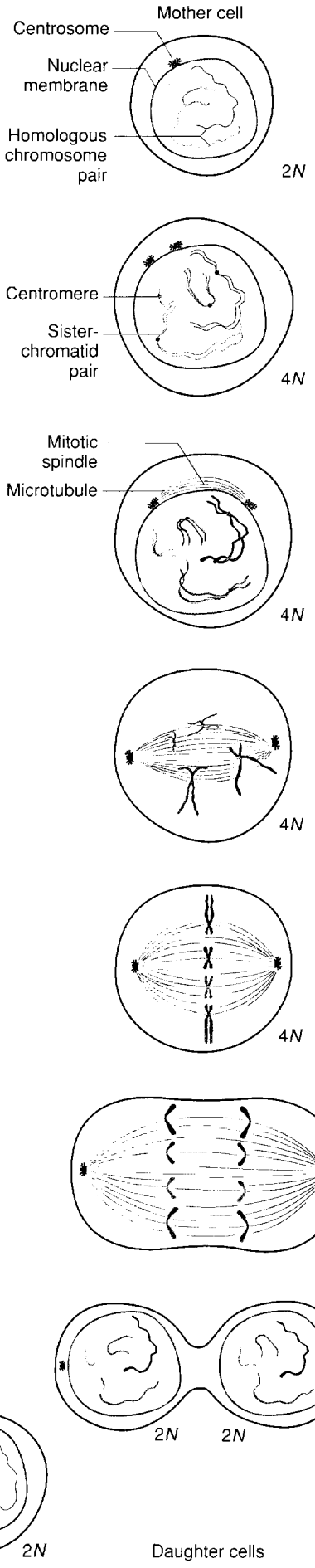
Meiosis, the type of cell division undergone by the precursors of gametes, was found to be a much more complex process than mitosis. It involves two successive cell divisions and can yield four gametes each containing one-half the number of chromosomes as the precursor cell. (Thus meiosis also must be preceded by chromosome duplication.) Furthermore, the haploid set of chromosomes in each gamete is not a haphazard selection from the diploid set of the mother cell. Instead each gamete is endowed with a randomly selected member of each pair of homologous chromosomes in the mother cell (see "Meiosis"). That is, the probability of a gamete's being endowed with one member of a pair of homologous chromosomes is the same as the probability of its being endowed with the other member, and, equally important, the outcome of its endowment with a member of one pair of homologous chromosomes has no effect on the outcome of its endowment with a member of another pair. In other (and more arcane) words, meiosis equally segregates each pair of homologous chromosomes and independently assorts the complete set of homologous chromosomes.

The X chromosome and the Y chromosome of a male also were found to segregate equally during meiosis, even though they are not homologous in the sense of being physically indistinguishable. That fact implies that a male produces two equally probable sperm types, one containing a Y chromosome and the other an X chromosome. Thus fertilization of an egg by a sperm results in two equally probable combinations of sex chromosomes, XY and XX.

The equal segregation and independent assortment of chromosomes during meiosis leads to diversity among the chromosome sets of the offspring of sexually reproducing organisms. Consider, for example, an organism that possesses but two pairs of homologous chromosomes denoted by 1 and 1′ and 2 and 2′. Such an organism produces, with equal probability, four types of gametes, those containing 1 and 2, 1 and 2′, 1′ and 2, and 1′ and 2′. If the organism is self-fertilizing (as are many plants and lower animals), then of the sixteen possible types of offspring, only four possess a set of chromosomes identical to the parental set. In contrast, bacteria reproduce asexually by a type of cell division that, like mitosis, yields only genetic replicas of the mother cell. (Bacteria are not, however, genetically immutable, since various mechanisms can effect changes in their genetic material, which are then transmitted to their offspring.) In general, if a sexually reproducing organism has N pairs of homologous chromosomes, it can produce 2^N types of gametes, and if it is self-fertilizing, only 2^N of the 2^{2N} possible types of offspring possess a set of chromosomes identical to the parental set. In other words, the probability of an offspring's possessing a set of chromosomes identical to the parental set is $1/2^N$. When N equals twenty-three, that probability equals 1/8,388,608, a very small number. The probability of human parents producing an offspring with a set of chromosomes identical to that of either parent is even closer to zero, since although humans do possess twenty-three pairs of equally segregating and independently assorting chromosomes, they are not of course self-fertilizing. Discussed later is a process that leads to even more differences among the chromosome sets of sexually

MITOSIS

Mitosis is the type of cell division that produces two daughter cells from a single mother cell. Each daughter cell has a set of chromosomes identical to the set possessed by the mother cell. Mitosis is the mechanism whereby a multicellular organism increases in size and replaces dead cells and whereby single-celled eukaryotic organisms reproduce asexually. The interested reader can find a striking series of photomicrographs of mitosis in the lily *Haemanthus katherinae* on page 7 of *Genes and Genomes: A Changing Perspective* by Maxine Singer and Paul Berg (University Science Books, 1991).



INTERPHASE

G_1—During G_1 (see "The Eukaryotic Cell Cycle") the chromosomes of the mother cell are very long and very thin. Only two of the cell's *N* pairs of homologous chromosomes are shown, and the members of each homologous pair are depicted in different shades of the same color. The centrosome is the source of fibrous proteins called microtubules. One function of microtubules is to direct the motion of chromosomes during mitosis (and meiosis).

G_2—The mother cell has replicated its complement of chromosomes (during the preceding S phase) and all other cellular material required for cell division, including the centrosome. The two identical copies of each chromosome are bound together along their centromeres into a so-called sister-chromatid pair.

MITOTIC PHASE

Prophase

The onset of mitosis is signaled by the ordered compaction, or condensation, of chromosomes into microscopically visible threads. Microtubules radiating from the two centrosomes collectively compose the mitotic spindle.

Prometaphase

The chromosomes have condensed further, and the centrosomes have migrated to opposite sides of the cell. Disintegration of the nuclear membrane has allowed microtubules to bind to each chromosome at a region within its centromere.

Metaphase

The chromosomes have assumed their most condensed configuration, and the sister-chromatid pairs have assumed the familiar X shape. Under the influence of opposing forces exerted by microtubules radiating from both centrosomes, each sister-chromatid pair has become aligned along the midplane of the cell.

Anaphase

The bond joining each sister-chromatid pair has broken, and the members of each former sister-chromatid pair have begun moving toward opposite sides of the cell. As a result, a set of chromosomes identical to the set initially possessed by the mother cell becomes segregated in each side of the cell. The cell has begun to elongate and narrow at the midplane.

Telophase

A new nuclear membrane has formed around each segregated set of chromosomes, the chromosomes have begun to decondense, and the cell has begun to divide.

INTERPHASE

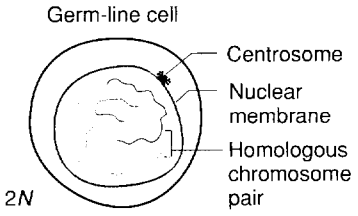
G_1—Cleavage of the extranuclear cellular material has produced two daughter cells, and the chromosomes in each have decondensed further in preparation for the biosynthetic activities of G_1.

MEIOSIS

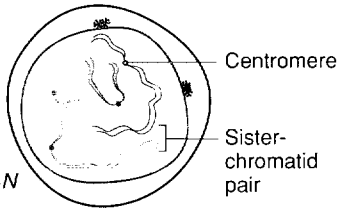
Meiosis is the type of cell division that produces the gametes (eggs and sperms) whose union is the first step in the creation of a new human or other sexually reproducing organism. Only so-called germ-line cells undergo meiosis, and each gamete contains a haploid set of chromosomes—a set composed of one member of each of the *N* pairs of homologous chromosomes possessed by the diploid germ-line cell. The transition from diploidy to haploidy is accomplished by two successive partitions of nuclear material. During each partition the motions of the chromosomes are directed, as they are during mitosis, by microtubules radiating from two centrosomes.

PREMEIOTIC PHASE

The germ-line cell, which may be an oogonium in an ovary or a spermatogonium in a testis, appears little different from a somatic cell in G_1. Only two of the germ-line cell's *N* pairs of homologous chromosomes are shown, and the members of each homologous pair are depicted in different shades of the same color.



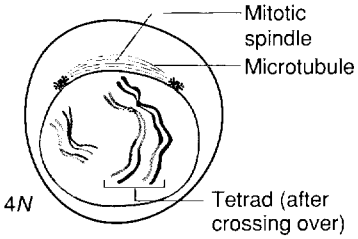
The germ-line cell has replicated its complement of chromosomes and all other cellular material required for cell division, including the centrosome. The two identical copies of each chromosome are bound together along their centromeres into a sister-chromotid pair.



MEIOTIC PHASE

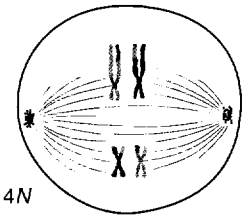
Prophase I

The onset of meiosis is signaled by a limited condensation of chromosomes. Homologous sister-chromatid pairs have become closely associated, forming *N* tetrads and allowing "crossing over" to occur, here within only one tetrad. Crossing over results in the exchange of corresponding portions of homologous chromosomes. The germ-line cell now lingers in prophase I for a time that ranges, depending on the species, from a few days to many years.



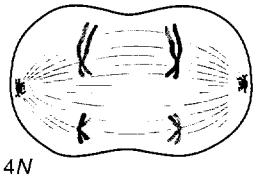
Metaphase I

The germ-line cell has passed through prometaphase I (not shown) and has entered metaphase I. The chromosomes have fully condensed, and the tetrads have become aligned along the midplane of the cell.



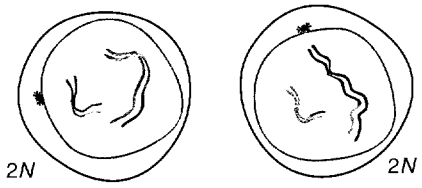
Anaphase I

The members of each tetrad have separated and begun moving toward opposite sides of the cell. Depicted here is but one of the 2^N possible outcomes of the motion of the members of the *N* tetrads. The equal probability of each possible outcome is the physical basis for Mendel's laws of equal segregation and independent assortment.



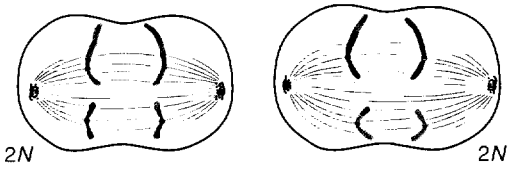
Prophase II

The germ-line cell has passed through telophase I (not shown) and has divided into two cells, each of which has entered prophase II. Note that the products of the first meiotic division, like the products of mitosis, have the same number of chromosomes as the original cell. However, a product of mitosis contains *N* homologous chromosome pairs, whereas a product of the first meiotic division contains two identical copies of each of *N* nonhomologous chromosomes.



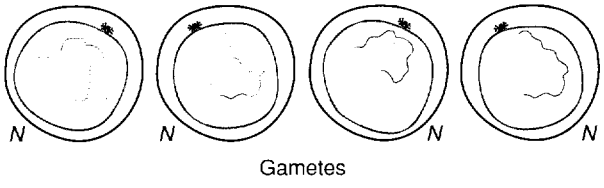
Anaphase II

Both cells have passed through prometaphase II and metaphase II (not shown). Each sister-chromatid pair has separated, and the members of each former sister-chromatid pair have begun migrating to opposite sides of the cell.



POSTMEIOTIC PHASE

Each cell has passed through telophase II (not shown) and divided into two gametes. Thus each meiosis can yield four gametes. However, meiosis of an oogonium usually yields only one egg because each division of extranuclear material usually yields only one cell that survives because it receives most of the extranuclear material.



reproducing organisms and their offspring: the "crossing over" that occurs between homologous chromosomes during the first stage of meiosis (see "Meiosis"). Together, crossing over and equal segregation and independent assortment essentially guarantee that in the whole history of *Homo sapiens*, no two individuals (except the pairs of identical twins arising from single fertilized eggs) have been alike genetically.

The facts that accumulated about chromosomes and their behavior during mitosis and meiosis suggested that the link between generations (of cells or organisms) was a substance present in chromosomes. In 1896 the American cell biologist Edmund Beecher Wilson (1856–1939) suggested that the substance of inheritance was the "nuclein" isolated in 1874 by the Swiss chemist Johann Friedrich Miescher (1844–1895) from the nuclei of human pus cells and salmon sperms. Nuclein was found to be composed of two types of chemicals, a nucleic acid and various "albumins," or proteins. By the end of the century, the most advanced thinkers about the mechanism of inheritance, such as Wilson, Boveri, and August Friedrich Leopold Weismann (1834–1915), were of the opinion that nuclein was the stuff of inheritance.

A Theory of Inheritance. The nineteenth century was the setting also for the elegant work of the Austrian Gregor Johann Mendel (1822–1884), an Augustinian monk better versed in mathematics and physics than in biology. In 1865 Mendel published visionary explanations for the results of his plant-breeding experiments. Among them was the notion that discrete units of heredity (which he called *Merkmale* and we call genes) are passed unchanged from generation to generation even though each unit is not necessarily expressed as an observable trait in every generation. He also proposed that each plant possesses two such units for each observable trait, one inherited from its male parent and the other from its female parent. Mendel developed statistical laws for predicting how the paired units of heredity are parceled out to offspring. The laws are now known to be applicable (within certain limits) to all sexually reproducing organisms. Furthermore, Mendel's laws parallel the behavior of homologous chromosome pairs during meiosis (the equal segregation of a single chromosome pair and the independent assortment of different chromosome pairs) because, as we now know, Mendel's units of heredity reside on chromosomes. Remarkably, Mendel deduced his theory *before* chromosomes were identified as the probable carriers of genetic information. His proposals are discussed here out of chronological order because their significance to the emerging science of genetics was not grasped—and probably could not have been grasped—until after the observed behavior of chromosomes during meiosis could provide a physical basis for his abstract theory. Mendel's publication remained unknown, in fact, until 1900 when, working independently, the German botanist Karl Erich Correns (1864–1933), the Dutch botanist Hugo De Vries (1848–1935), and the Austrian botanist Erich Tschermak von Seysenegg (1871–1962) performed similar experiments, arrived at similar explanations, and brought Mendel's publication to light, garnering him well-deserved albeit posthumous fame.

To best appreciate Mendel's work, one needs to know something about the successes and shortcomings of previous efforts at selective breeding of plants and animals. Selective breeding was certainly well under way in the Neolithic period, and numerous early successes produced most of the strains of domestic plants and animals now in existence. Some of the plant-breeding efforts led to plants so different from their ancestral relatives that they can be considered human-made species. Notable examples are today's *Zea mays* (maize, or corn) and *Solanum tuberosum* (the potato plant). Natives of present-day Mexico began developing maize from tiny-eared relatives between 4000 and 5000 years ago, and the pre-Columbian inhabitants of present-day Peru and Bolivia developed a plant producing palatable tubers from relatives producing tubers so bitter as to be inedible. When introduced into the Old World in the sixteenth century, maize and the potato had a tremendous influence on the world's economy. The potato, for example, replaced wheat and rye in the cool areas of northern Europe as a staple food because it produces more calories per acre. (Only rice is as efficient a calorie-producer as the potato, and rice is a warm-climate plant.) The introduction of maize and the potato is thought by some historians to have significantly accelerated the great increase in the rate of population growth of western Europe that began in about the fourteenth century.

Successful as the early breeding efforts were, and those of the noted eighteenth-century plant breeders Josef Gottlieb Koelreuter (1733–1806) and Joseph Gaertner (1732–1791), they certainly were not what we would now call scientific, since in general the outcomes of breedings were quite unpredictable. In contrast, Mendel's aim at the outset of his eight-year effort was to ascertain the statistical rules governing the inheritance of variable traits. Both his methodology and his theoretical conclusions are the foundation for all future studies in genetics.

Mendel chose to work with a plant that exhibits distinct variants of a number of traits, the garden pea (*Pisum sativum*). He concentrated on two variants of each of seven traits, including pod color (green and yellow) and flower color (violet and white). His unique experimental approach began by allowing plants that bore, say, green pods to self-pollinate for a sufficient number of generations to assure that each new generation of self-pollinated plants would also bear only green pods. Since each of the fourteen purebred strains consistently bore only one variant of each of a single trait, the purebred strains were advantageous to Mendel's work, providing a certain and observable starting point and amounting, essentially, to a control on his experiments. Mendel proceeded to study the inheritance of each of the seven traits, first one at a time and then in pairs. All of the experiments on the inheritance of single traits followed the same pattern as that described here for pod color.

First, Mendel cross-pollinated the two strains purebred for pod color, the strain bred true for green pods and the strain bred true for yellow pods. (Together the two purebred strains are called the parental generation.) Regardless of which strain he used as the male (pollen-contributing) parent, all the resulting offspring (called here hybrids or members of the first generation) bore only green pods. Today we would

say that all members of the first generation exhibited the same phenotype, a term introduced in 1909 by the Danish botanist Wilhelm Ludwig Johannsen (1857–1927). Symbolically,

$$\text{parental generation} \rightarrow \text{first generation},$$

and in particular,

$$\text{purebred green} \times \text{purebred yellow} \rightarrow \text{hybrids, all green.}$$

The natural question to ask is: Has the capacity to produce the yellow-pod phenotype disappeared altogether, or is it still present but somehow suppressed in the first-generation hybrids? To find out, Mendel selfed the hybrids (that is, he allowed them to self-pollinate), and he observed that the yellow-pod phenotype reappeared among the resulting offspring (the second generation). When Mendel counted the number of second-generation offspring exhibiting each phenotype (a novel procedure at the time), he found that the ratio of green-podded plants to yellow-podded plants was approximately 3 to 1. Symbolically,

$$\text{first generation} \rightarrow \text{second generation}$$

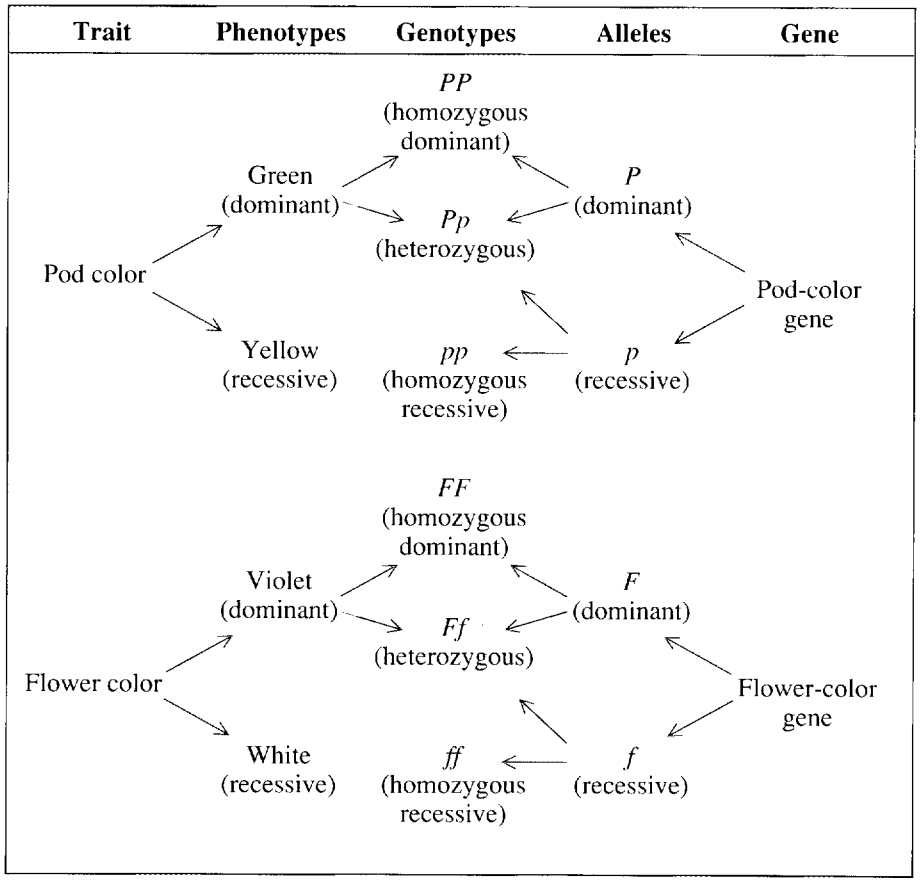
and in particular,

$$\text{green hybrid} \times \text{green hybrid} \rightarrow 3 \text{ green} : 1 \text{ yellow.}$$

To find out whether any members of the second generation had the capacity to produce offspring with the phenotype they themselves did not exhibit, Mendel selfed the members of the second generation. He found that all the yellow-podded members behaved like plants purebred for yellow pod color; that is, they produced only yellow-podded offspring. In contrast, only one-third of the green-podded members of the second generation behaved like plants purebred for green pod color, whereas the remaining two-thirds behaved like the first-generation hybrids, producing both green- and yellow-podded progeny in the ratio of 3 to 1. In other words, the ratio 3 green:1 yellow exhibited by the second generation is more accurately described as the ratio 1 pure green:2 hybrid green:1 pure yellow. Mendel continued selfing the green-podded members of successive generations and always found that approximately two-thirds of the green-podded progeny of green hybrids were again green hybrids, behaving just like the first-generation hybrids. That is, when those two-thirds were allowed to self-pollinate, they produced green- and yellow-podded progeny in the approximate ratio of 3 to 1.

To explain the mathematical regularity of his results, Mendel advanced a theoretical model of inheritance. First, and most basic, is the idea that the fertilized egg (zygote) from which a plant develops contains two genes, or units of heredity, for pod color, one contributed by the egg and the other contributed by the sperm. ("Gene" is another term coined by Johannsen.) Mendel also proposed that there were two distinct genes for pod color, one for green and one for yellow. The gene for green pod color he called dominant (and designated it by a capital letter, say P) because any plant that

carried that gene bore green pods. The gene for yellow pod color he called recessive (and designated it by a lowercase letter, *p*). Today we say *P* and *p* are different forms, or alleles, of the gene for pod color. Since the egg and sperm each contain only one allele, a fertilized egg contains one of three possible allele pairs (or possesses one of three possible genotypes, another word coined by Johanssen): *PP*, *Pp*, or *pp*. Mendel proposed that the plants purebred for green pod color contained the pair *PP*, those purebred for yellow pod color contained the pair *pp*, and the hybrid plants, which bore only green pods but produced both green- and yellow-podded progeny when allowed to self-pollinate, contained the pair *Pp*. In modern terminology plants possessing the genotype *PP* are said to be homozygous dominant; those possessing the genotype *pp* are homozygous recessive; and those with the genotype *Pp* are heterozygous. This terminology and other nomenclature of genetics is illustrated in the table.



With those hypotheses and the laws of probability Mendel constructed a probabilistic model that explained the results of his experiments. The model is shown in "Mendelian Genetics." The element of chance is operative in both the formation of gametes (eggs and sperms) and in the formation of zygotes (fertilized eggs). Mendel assumed that during the formation of gametes, the pair of alleles for pod color separates (or segregates) equally; in other words, the probability that a gamete will receive one or the other of the pair is equal to one-half. He therefore predicted correctly that among the gametes produced by a green hybrid (a plant heterozygous for pod color), approximately one-half would contain *P* and the remainder would contain *p*. Because, as is now known, each member of the allele pair for a given trait resides at the same location on one or the other of a pair of homologous, equally segregating chromosomes, only one allele enters each gamete. Therefore, the behavior of a single allele pair during meiosis is known as Mendel's law of equal segregation.

The element of chance is also operative in the random union of an egg and a sperm to form a zygote with a particular genotype. For example, in the formation of offspring of the green hybrids, the probability of forming a zygote with the genotype *PP*, call it Pr(*PP*), is the joint probability of two independent events, namely, the probability

that an egg contains P, and the probability that a sperm contains P. Since the joint probability is the product of the probabilities of the two independent events, we can write $\Pr(PP) = \Pr(P)\Pr(P)$.

Mendel applied this rule to predict the probability of finding a given genotype among the progeny of the green hybrids. Since green hybrids produce gametes containing P or p, each with a probability of 1/2, the eggs and sperms combine in four equally probable ways to produce offspring with the genotypes PP, Pp, pP, or pp, and the probability of each of those genotypes is 1/2 times 1/2, or 1/4. Since Pp and pP are equivalent genotypes (it doesn't matter whether a particular allele arrived with the sperm or the egg), the probabilities for Pp and pP are added to predict that the probability of an offspring's having the genotype Pp is 1/2. In other words, the three possible genotypes occur in the ratio 1 PP:2 Pp:1pp. Translating the genotypes into phenotypes yields the ratio 3 green:1 yellow in agreement with Mendel's observations.

Having explained the 3 green:1 yellow ratio by advancing a general model, Mendel went on to test the model by crossing green hybrids (genotype Pp) with plants purebred for yellow pod color (genotype pp). He predicted that the offspring would have the genotypes Pp and pp in the ratio 1 Pp:1 pp and found, in agreement with the model, that approximately one-half the progeny bore green pods and the remainder bore yellow pods.

Mendel obtained similar results for all seven traits. In other words, he inferred the existence of two alleles for each trait, one dominant and one recessive. However, we now know that the alleles of a gene do not always exhibit a dominant-recessive relationship. Sometimes the pairing of different alleles leads to a blend (for example, pairing of the snapdragon alleles that specify white and red flowers leads to pink flowers); sometimes it leads to simultaneous exhibition of both phenotypes (for example, pairing of the human alleles that specify A and B blood types, which are characterized by the presence of the antigens A and B, respectively, on the surface of red blood cells, leads to AB blood type, which is characterized by the presence of both antigens). However, the validity of Mendel's research and theoretical conclusions is unaffected by the fact that he focused, presumably by chance, on traits controlled by alleles that do exhibit the phenomenon of dominance.

Mendel next proceeded to study the co-inheritance of two traits, say pod color (specified by dominant and recessive alleles P and p, respectively) and flower color (specified by dominant and recessive alleles F and f, respectively). Again, he first developed two purebred strains, one purebred for green pod color and violet flower color (genotype $PPFF$) and the other purebred for yellow pod color and white flower color (genotype $ppff$).

As before, Mendel cross-pollinated the purebred strains, thus producing dihybrid offspring, each heterozygous for both traits. He selfed the resulting first dihybrid generation to produce the second dihybrid generation. Each member of the first

dihybrid generation exhibited both dominant phenotypes; that is, they bore green pods and violet flowers. Members of the second dihybrid generation exhibited four composite phenotypes in a 9:3:3:1 ratio, as shown below.

Possible Phenotypes among Second Dihybrid Generation	Fraction Exhibiting Phenotype
green pods, violet flowers	$\frac{9}{16}$
green pods, white flowers	$\frac{3}{16}$
yellow pods, violet flowers	$\frac{3}{16}$
yellow pods, white flowers	$\frac{1}{16}$

Note that the ratio of green- to yellow-podded members of the second dihybrid generation was still 3 to 1, just as it was in the second generation produced by the experiments on pod color alone. The ratio of violet- to white-flowered members of the second dihybrid generation also was 3 to 1. Mendel realized that the 9:3:3:1 ratio resulted from multiplicative combinations of the two 3:1 ratios. He therefore concluded that the phenotypes for the two traits are inherited independently. In other words, the probability of each composite phenotype is the product of the probabilities of the two "component" (single-trait) phenotypes. For example, the probability that a second-dihybrid-generation member will bear green pods and white flowers (3/16) is the product of the probability of its bearing green pods (3/4) and the probability of its bearing white flowers (1/4).

The independent inheritance of the two traits implies that when members of the first dihybrid generation produce gametes, segregation of the alleles for pod color is independent of the segregation of the alleles for flower color. In other words, the two allele pairs assort independently. The members of the first dihybrid generation have the genotype *PpFf*, so each gamete receives *P* or *p* with a probability of 1/2 and *F* or *f* with a probability of 1/2. Since the segregation of each allele pair is an independent event, the individual probabilities are multiplied to predict that the probability of forming each of the four possible types of gametes, those containing *PF*, *Pf*, *pF*, or *pf*, is 1/2 times 1/2, or 1/4.

Random fertilization of eggs by sperms produces the sixteen genotypes shown in the probability table for the second dihybrid generation in "Mendelian Genetics." Each has a probability of 1/4 times 1/4, or 1/16. The composite phenotype corresponding to each genotype is also shown. Counting the number of times each phenotype appears yields the 9:3:3:1 ratio observed by Mendel.

The physical basis for Mendel's law of independent assortment is the independent assortment of the various different pairs of homologous chromosomes during meiosis.

MENDELIAN GENETICS

Mendel's experiments on the inheritance of single traits and pairs of traits, illustrated here, led him to postulate the concept of discrete, particulate units of heredity that pass unchanged from generation to generation. He studied seven traits (characteristics) of the garden pea, each of which exhibited two alternative forms. For example, pod color could be either green or yellow, and flower color could be either violet or white. As described in the main text, Mendel found that one form of each trait was dominant and the other recessive and that the progeny of controlled breedings exhibited one form or the other in definite ratios. The observed mathematical regularities led to the model of inheritance described here. Mendel knew that his plants reproduced sexually, but he did not know that chromosomes exist nor that the number of chromosomes was reduced by one-half during the formation of gametes. As a result his terminology was rather imprecise. He did not clearly distinguish the form of a trait from the units of heredity whose actions determine the trait. That distinction was made almost half a century later by Johannsen, who coined the term gene for the particulate units of heredity, the term genotype for the genes whose action determines a trait, and the term phenotype for the form of the trait determined by the genotype. The more precise terminology is used in the following description of Mendel's model and in the accompanying figures.

Mendel's model of inheritance includes four postulates.

1. Each plant contains a pair of genes for each trait; that is, the genotype for a trait is specified by a pair of genes.

2. During the formation of gametes, the gene pair for a trait segregates equally; that is, the genes in the pair are parceled out to the gametes in a fashion such that each gamete receives only one member of the pair and has an equal chance of receiving either member of the pair (the law of equal segregation).

3. A gene has two forms, or alleles, designated by, say, *A* and *a*. Only plants with the genotype *aa* (homozygous for *a*) exhibit the recessive phenotype. A plant with the genotype *AA* (homozygous for *A*) or the genotype *Aa* (heterozygous) exhibits the dominant phenotype.

4. During the formation of gametes, segregation of the gene pair for any one trait is independent of the segregation of the other gene pairs. Consequently a plant heterozygous for two traits (genotype *AaBb*) produces gametes containing *AB, Ab*, *aB*, and *ab* with equal probability (the law of independent assortment). Note that the law of independent assortment holds only if the genes for the different traits are on different pairs of homologous chromosomes.

Mendel's laws of equal segregation and independent assortment can be applied in two ways. If one knows the genotypes of both parents, one can predict the probability of the genotype of a future offspring. Or, working backward, if one observes in existing offspring the approximate ratios of phenotypes predicted by Mendel's laws, one can often infer the genotypes of the parents, just as Mendel did.

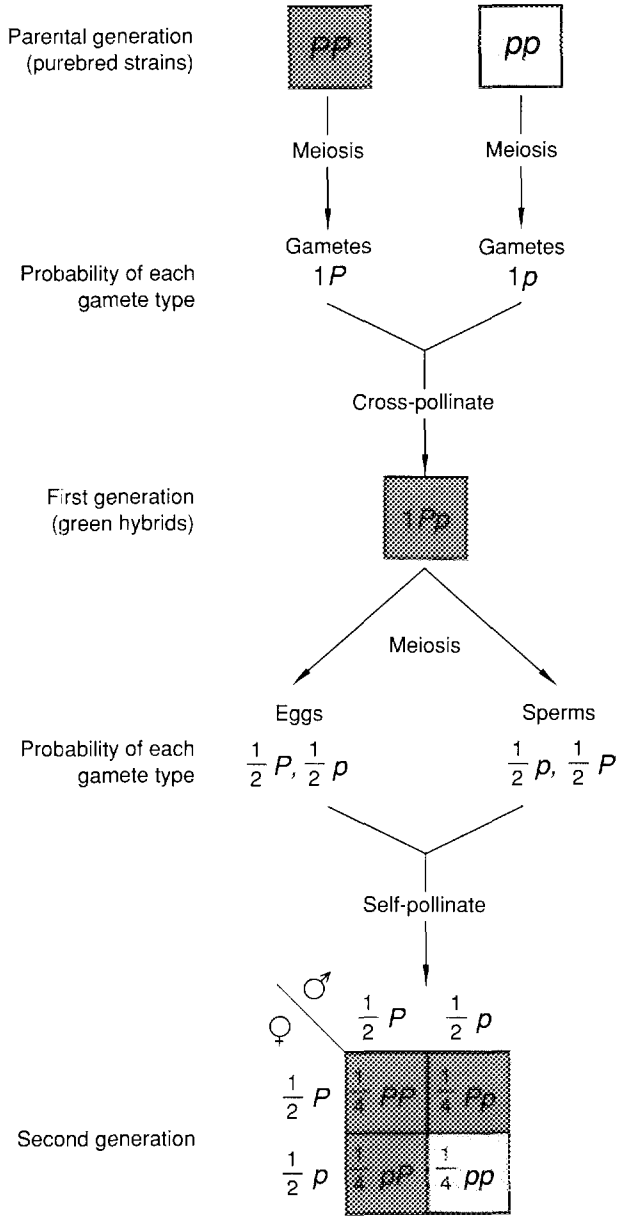
Mendel's Experiments on Inheritance of One Trait (Pod Color)

Methodology

Step 1: Cross-pollinate two strains of peas, one purebred for green pod color, the other purebred for yellow pod color. Result: All first-generation hybrids bear green pods.

Step 2: Self-pollinate the green hybrids. Result: Second-generation plants bear either green or yellow pods in the approximate ratio of 3 green to 1 yellow. Further selfing shows that half the second generation (or two-thirds of the green-podded members) are hybrids.

Theoretical Model



Mendel assumed that each plant contains a pair of genes for pod color. Therefore, each purebred parent is homozygous; that is, each contains two identical genes for pod color.

P = green-pod-color allele
p = yellow-pod-color allele

Since a fertilized egg results from the union of two gametes, each gamete contains one allele for pod color.

Because all first-generation offspring bore green pods, Mendel called green the dominant pod color and yellow the recessive pod color. Mendel inferred that whenever P, the allele for the dominant pod color, is present, the plant bears green pods (the law of dominance).

Mendel inferred that the pair Pp segregates equally into the gametes; that is, each gamete (whether egg or sperm) receives P or p with equal probability of one-half (law of equal segregation).

Random union of eggs and sperms produces four possible combinations of alleles in the offspring. As shown by the table, the probabilities of each gamete type are multiplied to yield the probabilities of the four possible genotypes in the second-generation offspring. Since Pp and pP are equivalent genotypes, the probabilities of each are added to yield a probability of one-half for the genotype Pp. Mendel's model predicts, for members of the second generation, phenotypes in the ratio 3 green : 1 yellow (in agreement with Mendel's observations) and genotypes in the ratio 1 PP : 2 Pp : 1 pp.

Mendel's Experiments on Inheritance of Two Traits (Pod Color and Flower Color)

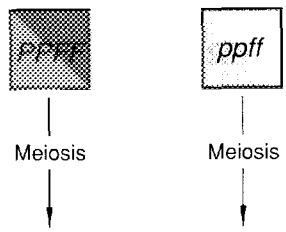
Methodology

Step 1: Cross-pollinate two strains of peas, one purebred for the two dominant phenotypes (green pods and violet flowers), the other purebred for the two recessive phenotypes (yellow pods and white flowers). Result: All first-generation dihybrids bear green pods and violet flowers.

Step 2: Self-pollinate the first-generation dihybrids. Result: Second-generation plants exhibit four composite phenotypes (pod color, flower color) in the ratio of 9 (green, violet) : 3 (yellow, violet) : 3 (green, white) : 1 (yellow, white).

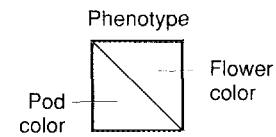
Theoretical Model

Parental generation (strains purebred for two traits)



Each purebred parent is homozygous for both pod color and flower color.

P = green-pod-color allele
p = yellow-pod-color allele
F = violet-flower-color allele
f = white-flower-color allele



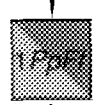
Probability of each gamete type

Gametes $1PF$ | Gametes $1pf$

Each gamete carries only one gene for each trait.

Cross-pollinate

First generation (green-pod and violet-flower dihybrids)



All first-generation (dihybrid) offspring bear violet flowers and green pods, the dominant phenotypes, in agreement with the law of dominance.

Meiosis

Probability of each gamete type

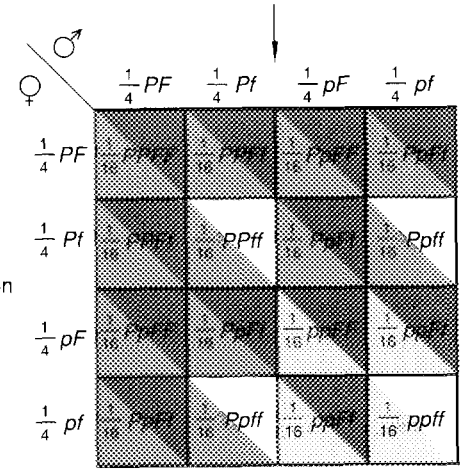
Eggs: $\frac{1}{4}PF,\ \frac{1}{4}Pf,\ \frac{1}{4}pF,\ \frac{1}{4}pf$

Sperms: $\frac{1}{4}PF,\ \frac{1}{4}Pf,\ \frac{1}{4}pF,\ \frac{1}{4}pf$

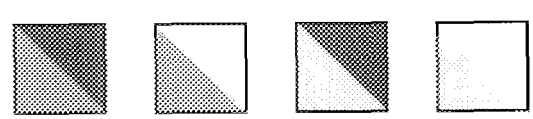
Independent equal segregation of each allele pair (*Pp* and *Ff*) produces gametes containing one of four equally probable combinations of alleles (law of independent assortment).

Self-pollinate

Second generation



Random union of eggs and sperms produces offspring containing one of sixteen equally probable combinations of alleles. All are equally probable because all gamete types are equally probable. The sixteen combinations reduce to nine different genotypes and four different composite phenotypes, which are predicted from the probability table to occur in the ratio 9:3:3:1 in agreement with Mendel's observations.



9 : 3 : 3 : 1

Therefore, the law applies *only* if the allele pairs for the two traits reside on different pairs of homologous chromosomes. In fact, deviations from Mendelian predictions for the co-inheritance of two traits is evidence that the two traits are specified by allele pairs that reside on the same pair of homologous chromosomes.

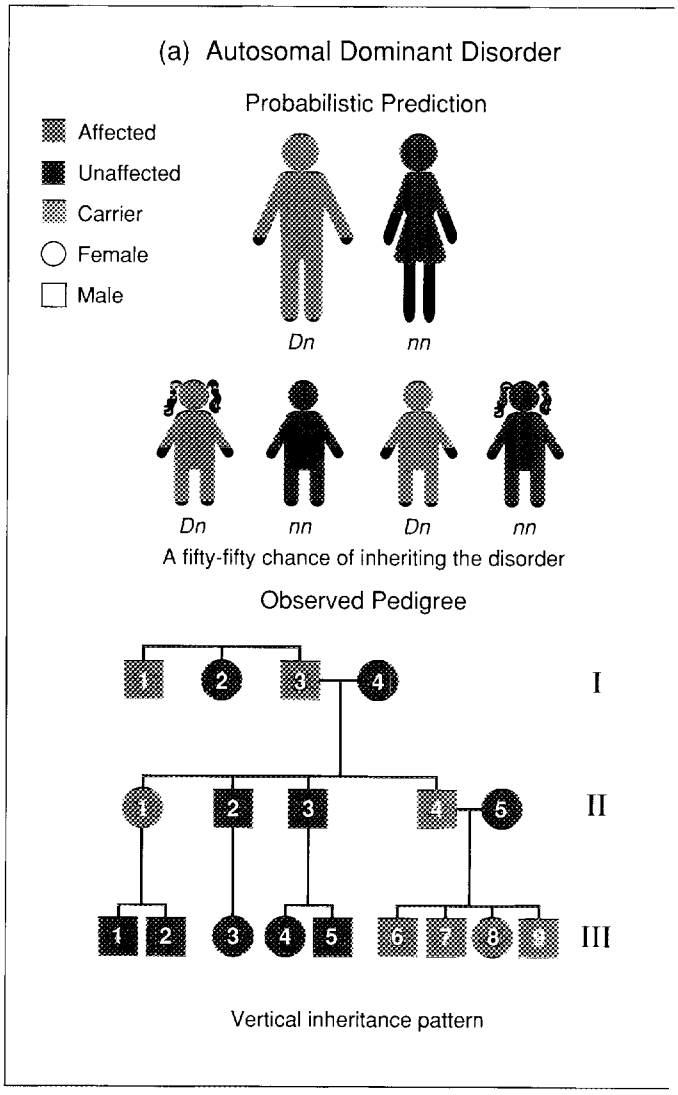
This discussion of Mendel's theory of inheritance ends with two points of note. First, although the theory is now known to be applicable to humans as well as to pea plants, it is unlikely that it could have been deduced from data about the outcomes of human breedings. As subjects of inheritance studies, humans pose several disadvantages: The controlled breeding of humans is generally regarded as inappropriate and would be difficult to achieve even if it were not; each pair of human parents typically produces too few data (offspring) for analysis of the sort required; and the rate at which humans produce offspring is too slow to suit most experimenters' taste. Moreover, many human traits are specified not by a single allele pair but by many allele pairs.

The second point of note concerns the utility of Mendel's theory as a predictive tool, particularly for human breedings. The theory can be applied directly only to traits determined by a single allele pair. Such traits are called Mendelian traits because they are inherited in accordance with Mendel's laws. Most Mendelian traits of humans are disorders—some mild, some grave—caused by the presence of a defective allele. To determine the probability that an offspring will be affected by a Mendelian disorder requires knowing the parental genotypes for the disorder and whether the disorder is caused by a dominant or a recessive allele. The required genotypic information for the parents can often be inferred from the phenotypes of their existing offspring and of their parents, and information about whether the defective allele is dominant or recessive can often be inferred from the pattern of inheritance of the disorder in other families (see "Inheritance of Mendelian Disorders"). More than three thousand human Mendelian disorders have been identified. One of the goals of the Human Genome Project is to supply the tools necessary to isolate the causative alleles from the vast quantity of human genetic material and to identify the defects in the alleles.

A Theory of Evolution. The nineteenth century brought not only the rise of cell biology and the work of Mendel but also a growing acceptance of the fact of evolution, of the creation of extant organisms by changes in the life forms that first populated this planet. Belief in the ancient principle of the invariability of species waned, and in its place came the conviction that new species had been and are being formed. (A notable holdout to the idea of evolution was the eminent Harvard zoologist Jean Louis Rudolphe Agassiz (1807–1873), who was what we would today call a creationist.) The veering of scientific opinion toward evolution led to development of a theory of evolution based on natural selection. Formulated independently by Charles Robert Darwin (1809–1882) and Alfred Russell Wallace (1823–1913), the theory was presented to the world first in a jointly authored short publication (1858) and later in Darwin's classic book *On the Origin of Species* (1859). Crucial to development of the theory were the observations that offspring resembled their parents

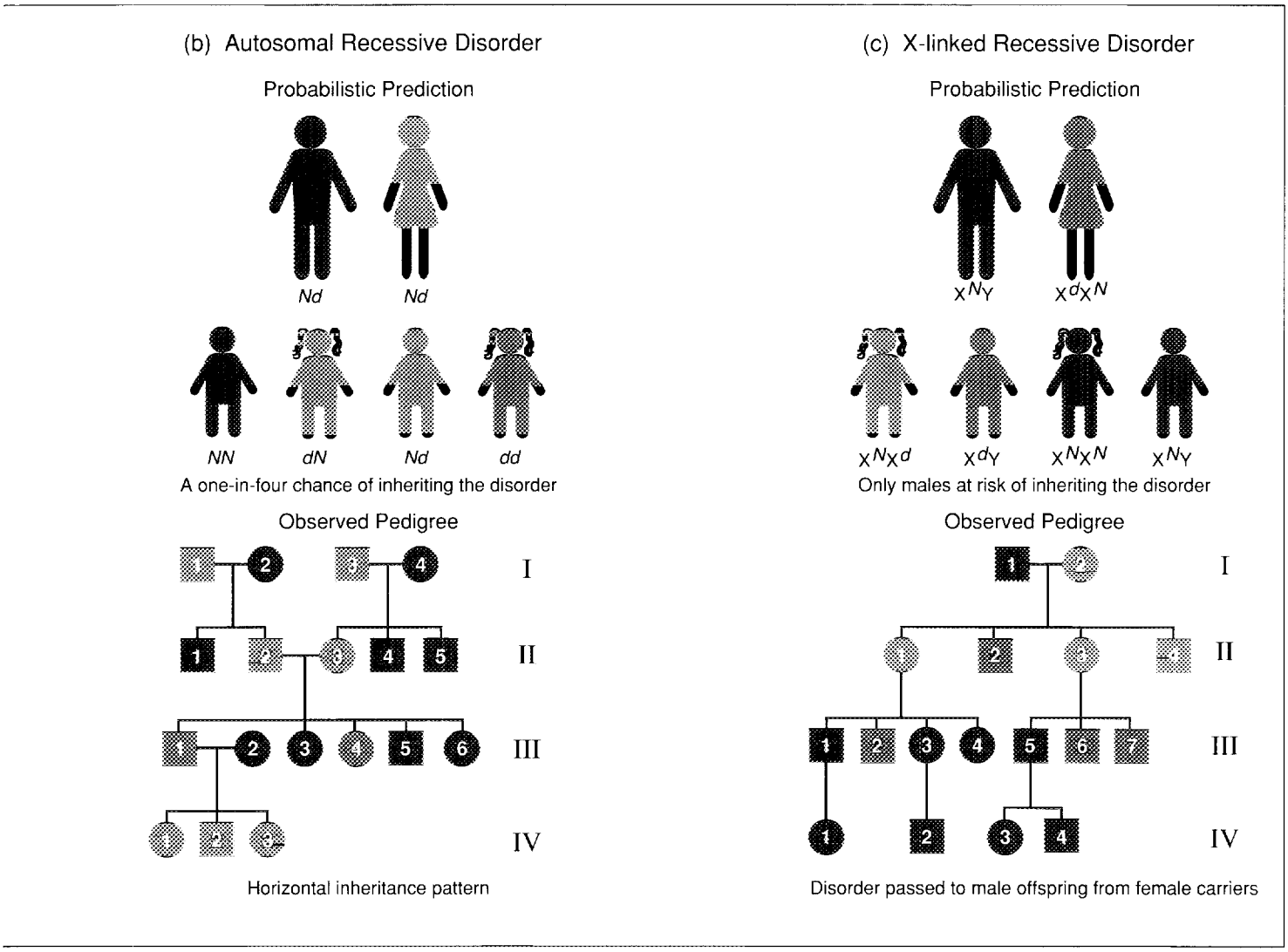
INHERITANCE OF MENDELIAN DISORDERS

Although some inherited disorders of humans are due to the combined effects of multiple genes (multigenic disorders) or to the combined effects of genes and the environment (multifactorial disorders), a so-called Mendelian disorder is caused by a single defective allele. Over 3000 Mendelian disorders are known. They range from mild conditions such as red-green color blindness to life-threatening diseases such as cystic fibrosis. Because the defective allele can be either dominant or recessive and can reside on either an autosome or a sex chromosome (in particular, the X chromosome–very few genes reside on the small human Y chromosome), four types of Mendelian disorders are possible: autosomal dominant, autosomal recessive, X-linked dominant, and X-linked recessive. Each type of disorder reveals itself through a distinctive pattern of inheritance in a family pedigree. Illustrated here are the patterns for three of the four types of Mendelian disorders.



Consider first the inheritance of an autosomal dominant Mendelian disorder. Many such disorders are expressed only in adulthood, including Huntington's disease, neurofibromatosis, and polycystic kidney disease. Shown in (a) are the equally probable genotypes and the phenotypes of the offspring of an affected father and an unaffected mother (or of an affected mother and an unaffected father). The genotype of the affected father can be either *DD* or *Dn*, where *n* is the nondefective recessive version of the defective dominant allele *D*. Because the father's having the genotype *DD* is the less typical and less interesting situation (all his offspring would be affected), it is assumed in (a) that the father has the genotype *Dn*. Because the mother is unaffected, her genotype must be *nn*. The equal segregation of chromosomes during meiosis implies that the offspring of such a mating can have one of two equally probable genotypes: *Dn* or *nn*. Therefore the probability of an offspring's being affected is 1/2. Note carefully, though, that only in the limit of an infinite number of offspring will the ratio of affected to unaffected offspring be equal to 1. Also shown in (a) is the pedigree of a family afflicted with hypercholesterolemia, a dominant disorder that causes excess levels of cholesterol in the blood. A thirty-year-old white male (II-4) suffered a myocardial infarction, a type of heart blockage, and was then found to test positively for hypercholesterolemia. Further tests indicated that his sister (II-1) and his four children (III-6, III-7, III-8, III-9) also had hypercholesterolemia. In addition, a family history revealed that the man's father (I-3) and uncle (I-1) both died of myocardial infarctions before reaching the age of fifty-five. Note that all of II-4's children are affected by the disorder, an outcome that is not inconsistent (although it may appear to be) with the probabilistic predictions based on the chromosome theory of heredity. Note also that the disease appears in all three generations of the pedigree; such a "vertical" pattern is characteristic of dominant disorders.

Shown in (b) is the inheritance of an autosomal recessive Mendelian disorder, examples of which include Tay-Sachs disease, cystic fibrosis, and sickle-cell anemia. Assume a typical situation: Both parents are carriers, or, in other words, are unaffected but have the genotype *Nd*, where *N* is the nondefective dominant version of *d*. The equal segregation of chromosomes during meiosis implies that the probability of an offspring's having the genotype *dd* and therefore of being affected is 1/4. In addition, the probability of an offspring's having the genotype *Nd* or *dN* (and of being a



carrier) is 1/2 and of having the genotype *NN* (and of being unaffected) is 1/4. Also shown in (b) is the pedigree of a family with an autosomal recessive Mendelian disorder. Only two individuals, both in the third generation (III-1 and III-4), are affected. All the other individuals listed are either carriers or unaffected. Since typically siblings in only a single generation are affected by a recessive Mendelian disorder, its inheritance pattern is referred to as horizontal.

Shown in (c) is the inheritance of an X-linked recessive Mendelian disorder. Such disorders include hemophilia, which is the result of a lack of an essential blood-clotting factor, and Duchenne muscular dystrophy, which causes progressive muscle weakness and death in early adulthood from respiratory problems. Again assume a typical situation: The mother is a carrier and therefore has the genotype X^dX^N, and the father is unaffected and therefore has the genotype X^NY. Any male offspring has a probability of 1/2 of being affected, and any female offspring has a probability of 1/2 of being a carrier. Also shown in (c) is a pedigree of a family with Duchenne muscular dystrophy. One son (II-2) and two daughters (II-1 and II-3) inherited the maternal X chromosome on which the defective allele resides. The son, possessing only one X chromosome, is affected. On the other hand, the daughters are unaffected carriers, but their sons (III-2, III-6, and III-7) inherited the defective allele. The pedigree illustrates the typical pattern of inheritance of an X-linked recessive disorder: transmission from an affected male through his daughters to his grandsons. Females can inherit the disease if the father is affected and the mother is either affected or a carrier.

only incompletely and that selective breeding had produced plants and animals quite different from the ancestral strains. Darwin arrived at his conclusions in large part by doing a *Gedankenexperiment*, much as Albert Einstein later arrived at his theory of relativity. It should be noted that not all of Darwin's thinking was as forward-looking as his theory of evolution. He was an exponent of a form of pangenesis (see "Early Ideas about Heredity") and of blending inheritance (the notion that the characteristics of offspring are the result of a melding of the parental characteristics). Darwin's cousin Francis Galton (1822–1911), in his own way also a genius, tried to point out to Darwin, without success, that neither theory of inheritance made much sense. In doing so Galton came very close to developing the same theory of particulate inheritance as had Mendel, although like Darwin, he was unaware of Mendel's work. Like Mendel, Galton was cognizant of probability and statistics. He can be considered the founder of modern biostatistical theory, which has been an immensely powerful tool in the development of genetic theory.

The cell biologists, Mendel, and Darwin and Wallace made basic contributions to the foundations of modern genetics, but they did so essentially in isolation from each other. Mendel was influenced to some extent by the findings of the cell biologists and of the evolutionists, but neither of the latter were influenced by him or by each other. Such isolation among different fields of science, though detrimental to progress, is still today not uncommon.

Things Come Together

The science of genetics was born in the first decade of the twentieth century through fusion of Mendel's theory of inheritance and the cell biologists' knowledge about chromosomes. In 1902 a student of Wilson's, Walter Stanborough Sutton (1877–1916), and Boveri independently recognized the parallels between the real objects called chromosomes and the theoretical constructs called genes—the occurrence of both as pairs, their separation in a similar fashion during gamete formation, and their re-pairing during fertilization—and proposed that each member of a pair of alleles is located on one or the other member of a pair of homologous chromosomes. Thus was born the chromosome theory of heredity. The theory was soon proved, and during the period between 1910 and 1940—the heyday of classical genetics—many allele pairs were localized to particular homologous chromosome pairs.

Classical Genetics. The term "classical genetics" refers to those aspects of genetics that can be studied without reference to the molecular details of genes. The early stars of classical genetics were the American Thomas Hunt Morgan (1866–1945), his students Calvin Blackman Bridges (1889–1938), Hermann Joseph Muller (1890–1967), and Alfred Henry Sturtevant (1891–1970), and last but not least members of the genus *Drosophila*, most notably the common fruit fly *Drosophila melanogaster*. Morgan's interest lay (initially at least) in determining whether the changes that result in new species occur gradually or abruptly. He chose to study changes in *D. melanogaster*

because it reaches sexual maturity so rapidly, produces so many offspring, and is so easily and cheaply raised in the laboratory. The discovery, in the spring of 1910, of a lone white-eyed male fly among thousands upon thousands of red-eyed flies in the Fly Room at Columbia University was a momentous event, leading not only to proof of the chromosome theory of heredity but also to knowledge of previously unknown aspects of meiosis.

Now is an appropriate time to emphasize the critical role of mutants in genetics. (A mutant is a member of a species that exhibits a phenotype different from the "wild-type" phenotype exhibited by most members of a natural population of the species.) Even knowledge of the existence of a gene is usually inferred from the existence of a mutant. When faced, for example, with a vast population of only red-eyed flies, how could anyone suspect that eye color is a manifestation of genes in operation? To be discussed later is another invaluable role of mutants—as tools for learning more specifically what genes do. (That genes determine physically observable traits is certainly true but remarkably vague.)

An early outcome of the discovery of the white-eyed fly was Morgan's proposal that alleles for red and white eye color in *D. melanogaster* are located on its X chromosomes. Morgan arrived at that proposal by observing the eye colors of the progeny resulting from a series of breedings, a series that began with matings between the white-eyed male and wild-type red-eyed females. (Note that mutants must not only be discovered but also be allowed to survive and breed.) Because all the progeny were red-eyed, Morgan concluded that the red-eye-color allele is dominant. Next he interbred the progeny and found, just as Mendel would have predicted, that three-quarters of the resulting second-generation progeny were red-eyed and one-quarter were white-eyed. However, among neither the red-eyed nor the white-eyed second-generation flies did he find an equal number of males and females, as would be predicted if the observed segregation of sex chromosomes was independent of the presumed segregation of red-and white-eye-color alleles. Instead two-thirds of the red-eyed second-generation flies were females and all of the white-eyed flies were males. Morgan continued by mating red-eyed males to white-eyed females, a breeding that is the "reciprocal" of the original breeding of the lone white-eyed male. He found that half of the progeny were female and red-eyed and the other half were male and white-eyed, whereas Mendel would have predicted that all of the progeny would be red-eyed, just as all of the progeny resulting from the original breeding were red-eyed. To explain those deviations from Mendelian predictions, Morgan proposed that the red- and white-eye-color alleles are X-linked, or in other words that they are located on the X chromosomes.

The reader can more easily verify that Morgan's hypothesis explains the outcomes of the breedings he carried out by using some symbolism. Let w and W denote, respectively, the recessive white-eye-color allele and the dominant red-eye-color allele. Denote an X chromosome containing w by X^w and an X chromosome containing W by X^W. Then the first breeding Morgan carried out, the breeding

between wild-type red-eyed females and the white-eyed male, is denoted by $X^W X^W \times X^w Y$. The progeny of such a breeding contain one of two equally probable combinations of sex chromosomes: $X^W X^w$ and $X^W Y$. In other words, half the progeny are female and red-eyed and half are male and red-eyed. The reader is urged to verify that Morgan's proposal explains the outcomes of the other breedings he carried out, namely $X^W X^w \times X^W Y$ and $X^w X^w \times X^W Y$.

Morgan's experiments certainly supported the chromosome theory of heredity, but the work of Bridges provided more direct confirmation. Bridges started by repeating, on a large scale, one of the breedings Morgan had carried out, the breeding between white-eyed female flies and red-eyed male flies. If, as Morgan proposed, the w and W alleles reside on the X chromosomes, that breeding can be represented by $X^w X^w \times X^W Y$ and, as Morgan had observed, half of the resulting progeny would possess the sex-chromosome combination $X^w X^W$ (would be red-eyed females) and half would possess the sex-chromosome combination $X^w Y$ (would be white-eyed males). But Bridges' large-scale breeding produced a surprise: A very small fraction of the progeny (about one in every two thousand) were either white-eyed females or sterile red-eyed males. Bridges found, by direct microscopic observation of the chromosomes of the unusual progeny, that they possessed an anomolous number of sex chromosomes. The white-eyed females possessed two X chromosomes and one Y chromosome, and the sterile red-eyed males possessed a single X chromosome. Obviously the single X chromosome of a sterile red-eyed male must be the residence of the red-eye-color allele he must possess, and the pair of homologous X chromosomes of a white-eyed female must be the residences of the two white-eye-color alleles she must possess. Thus a combination of cytological data and genotypic and phenotypic data directly confirmed the chromosome theory of heredity. (Note that Bridges' "cytogenetic" evidence also indicated that the Y chromosome of *D. melanogaster* is involved in determining fertility rather than maleness.)

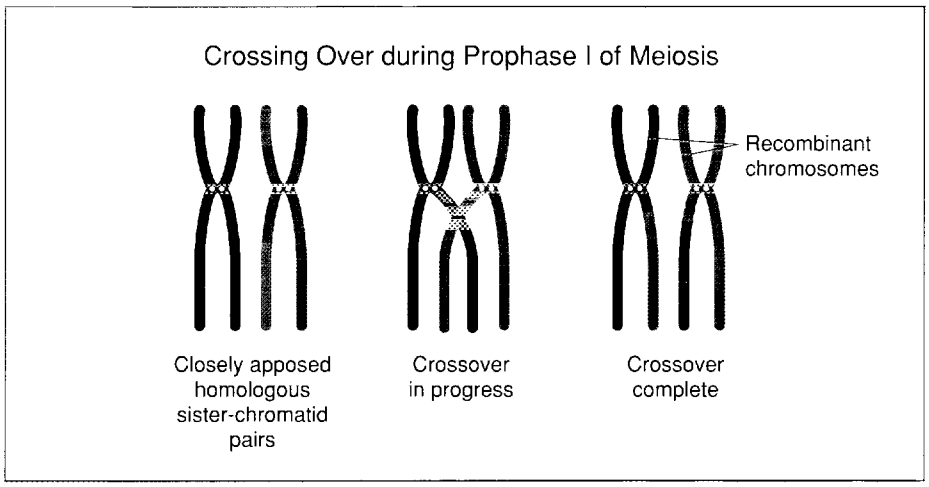
A question about Bridges' work remains: How could the abnormal numbers of sex chromosomes in the unusual progeny be explained? Bridges proposed that the homologous X chromosomes of a female fruit fly occasionally fail to segregate during meiosis. Meioses in which such "nondisjunctions" occur would yield two equally probable types of eggs: eggs containing two X chromosomes and eggs containing no X chromosomes. Fertilization of those two types of eggs by the two types of sperms produced by a male fruit fly would result in four types of fertilized eggs: those containing the combination of sex chromosomes $X_m X_m X_p$, the combination $X_m X_m Y$, the combination X_p, and the combination Y. (The subscript on each X chromosome denotes maternal origin or paternal origin.) The combinations $X_m X_m Y$ and X_p are the combinations Bridges observed in the unusual progeny; he attributed the absence of unusual progeny containing the $X_m X_m X_p$ and Y combinations to a lethal overdose and a lethal underdose of X chromosomes. Nondisjunction is now known to be a rare but medically significant feature of meiosis. The human disorder known as Down syndrome, for example, is caused by nondisjunction of chromosomes 21.

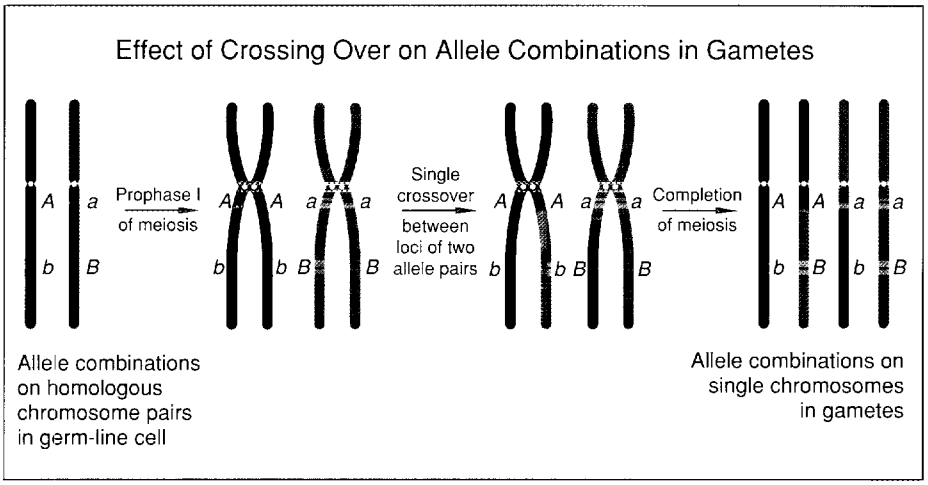
It is odd that proof for the existence of a rare meiotic glitch—nondisjunction—antedated clear evidence for the existence of what is now known to be a common feature of meiosis—crossing over. (Nondisjunction occurs once in about every hundred thousand human meioses, whereas crossing over occurs about thirty-three times per human meiosis, or on average more than once per homologous chromosome pair per human meiosis.) As proposed by Morgan, crossing over brings about an exchange, between two homologous chromosomes, of corresponding regions of the chromosomes. (An analogy is the exchange, between two nearly identical yardsticks, of, say, initial seven-inch regions.) Because homologous chromosomes differ from each other in details of their chemical composition, the products of a single crossover are two "recombinant" chromosomes, each different from (but still homologous to) the other and the chromosomes that participated in the crossover. In particular, if the exchanged regions contained different alleles of two genes, the recombinant chromosomes contain combinations of alleles that are different from the combinations of alleles possessed by the participants (see "Crossing Over: A Special Type of Recombination"). Thus crossing over, like independent assortment, increases the genetic diversity of sexually reproducing organisms. But whereas independent assortment merely creates new combinations of existing chromosomes, crossing over can create new chromosomes, ones containing new combinations of alleles.

Crossing over might today be regarded as merely another item in the phenomenology of meiosis were it not that it is the key element of a method for determining a measure of the distance between two genes (or, more precisely, two allele pairs) resident on the same chromosome (or, more precisely, on the same homologous chromosome pair). (Note that the method is applicable only to genes for which two or more alleles exist.) Called classical linkage analysis, the method is far from straightforward. The first step, of course, is to establish that two allele pairs are linked (are resident on the same homologous chromosome pair) by observing deviations from Mendelian predictions for the co-inheritance of the traits specified by the allele pairs. The next step is to measure the fraction of meioses in which crossing over leads to new combinations of alleles. The final step (and one not known to be necessary to the earliest linkage analysts) is to convert the measured "recombination fraction" to a "genetic distance" for the two allele pairs, which is defined as the probability of the occurrence of crossing over anywhere in the chromosomal region between the allele pairs. (Although a genetic distance is a dimensionless number, it is expressed in terms of a unit called a morgan or, more usually, in centimorgans.) The relationship between recombination fraction and genetic distance is complex (see "Classical Linkage Mapping" in "Mapping the Genome"), but a recombination fraction is approximately equal to its corresponding genetic distance when the recombination fraction is less than about 0.10. The significance of the genetic distance for two allele pairs is that the genetic distance is proportional to the physical distance between the loci of the allele pairs, provided crossing over occurs with equal probability at any point along the chromosome pair. Despite the fact that the stated proviso is not in general satisfied, genetic distance was until recently the only available measure of the physical distance between gene loci.

CROSSING OVER: a special type of recombination

DNA molecules, and hence chromosomes, are not immutable, even in the absence of external mutagenic agents. One of the natural mechanisms whereby DNA molecules can change is recombination, which rearranges genetic material by breaking and joining portions of the same DNA molecule or portions of different DNA molecules of the same organism. (Recombination can occur also between the DNA of an organism and the DNA of a virus that infects the organism.) Crossing over is the type of recombination undergone by the similar DNA molecules within two homologous chromosomes. It occurs almost exclusively during prophase I of meiosis, when homologous chromosomes are closely apposed. A single crossover between homologous chromosomes effects an exchange of corresponding chromosome regions and results in the formation of recombinant chromosomes, which differ in their content of hereditary information from the chromosomes that participated in the crossover. Crossing over also occurs between the identical DNA molecules within the chromosomes of a sister-chromatid pair, but because the recombinant chromosomes so formed are usually identical to the participants, such recombination has little genetic significance.





The occurrence of a single crossover between the loci of two allele pairs, say *A* and *a* and *B* and *b*, resident on a homologous chromosome pair results in the formation of some gametes that possess combinations of alleles different from the combinations possessed by the parent germ-line cell. Crossing over is thus a mechanism for increasing genetic diversity. It also is the basis of a standard method for determining a "distance" between the locus of *A* and *a* and the locus of *B* and *b*. The first step in the method (see "Determining a Genetic Distance") is to carry out a certain breeding experiment and thereby measure, among a group of gametes produced by one parent, the fraction possessing the new allele combinations (the so-called recombination fraction). When the measured recombination fraction is relatively small (less than about 0.10), it is approximately equal to the "genetic distance" between the two loci, that is, to the average number of crossovers between the two loci per meiosis. The genetic distance between the two loci in turn is a rough measure of the physical distance (the distance along the DNA molecule) between the two loci.

As illustrated in "Determining a Genetic Distance," linkage analysis is facilitated by carrying out either one of two particular breedings. (Each breeding is a "test cross" involving one doubly heterozygous parent and one doubly recessive parent.) Morgan happened to carry out both breedings—between fruit flies, of course—in the early 1910s and thereby not only gathered the first clear evidence for the existence of crossing over but also measured the first recombination fractions.

Then in 1913 Sturtevant measured recombination fractions for various pairwise combinations of six allele pairs known to reside on the X chromosomes of *Drosophila*. By assuming that the loci of the six allele pairs dot the X chromosome as points dot a line and that the measured recombination fraction for, say, the allele pairs *A,a* and *B,b* is directly proportional to the length of the X-chromosome segment between the locus of *A,a* and the locus of *B,b*, Sturtevant constructed a diagram—the first "genetic-linkage map"—showing the relative locations of the six genes and their pairwise separations. Sturtevant then used his diagram to calculate the recombination fractions for those pairwise combinations of allele pairs that he had measured but not needed to construct the diagram. The approximate agreement between calculated and measured recombination fractions indicated that both of his assumptions were at least approximately valid. We now know that, although the genes of all eukaryotic organisms lie along linear DNA molecules, the genes of prokaryotic organisms lie instead along circular DNA molecules. Furthermore, as indicated above, recombination fractions are not in general proportional to physical distance.

As noted previously, genetic studies of an organism demand the availability of mutants, that is, of individuals possessing alleles different from those possessed by wild-type individuals. For many years, though, geneticists had to survive on the rare mutants provided by nature. (Fewer than ten out of every million members of a natural population of a species are phenotypically obvious mutants.) Then in 1927 Muller (one of Morgan's trio of brilliant students) demonstrated that x rays induce heritable mutations in the fruit fly, and a year later the American geneticist Lewis John Stadler (1896–1954) used x rays to create new alleles in barley. The availability of x-ray-induced mutants accelerated the pace of gene discovery and genetic-linkage mapping.

The demonstrated power of combining cytological data about the chromosomes of an organism with genotypic and phenotypic data led, in the 1930s, to emergence of cytogenetics as a separate field of biology. Crucial to cytogeneticists is the ability to distinguish one pair of homologous metaphase chromosomes from another. For distinguishing features, early cytogeneticists relied on sizes and shapes, which do not always provide unambiguous identification. (The word "shape" generally means centromere location, but it can also mean an unusual structural feature specific to only certain metaphase chromosomes of certain organisms. Chromosome 9 of a strain of *Zea mays*, for example, is sometimes blessed with a conspicuous knob at the end of its short arm, a feature that helped elucidate the mechanism of crossing over.) It was soon learned, however, that each homologous chromosome pair within a metaphase