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Jurgen Schulz-Schaeffer

Cytogenetics

Plants, Animals, Humans

With 219 Figures

Springer-Verlag
New York Heidelberg Berlin

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Library of Congress Cataloging in Publication Data
Schulz-Schaeffer, Jurgen
Cytogenetics plants, animals, humans
Bibliography p
Includes index
1 Cytogenetics I Title
QH430.S38 1980 574 87'3223 79-25520

ISBN 0-387-90467-0
Springer-Verlag New York Heidelberg Berlin
ISBN 3-540-90467-0
Springer-Verlag Berlin Heidelberg New York

Preface

Since 1961 the author has taught a course in Cytogenetics at Montana State University Undergraduate and graduate students of Biology Chemistry Microbiology Animal and Range Science, Plant and Soil Science, Plant Pathology and Veterinary Science are enrolled Therefore the subject matter has been presented in an integrated way to correlate it with these diverse disciplines This book has been prepared as a text for this course The most recent Cytogenetics text was published in 1972, and rapidly developing research in this field makes a new one urgently needed

This book includes many aspects of Cytogenetics and related fields and is written for the college student as well as for the researcher It is recommended that the student should have taken preparatory courses in Principles of Genetics and Cytology The content is more than is usually taught during one quarter of an academic year, thus allowing an instructor to choose what he or she would like to present to a class This approach also allows the researcher to obtain a broad exposure to this field of biology References are generously supplied to stimulate original reading on the subject and to give access to valuable sources The detailed index is intended to be of special assistance to researchers

Individual chapters were carefully reviewed and constructively criticized by Drs PENELOPE ALLDERDICE (Memorial University, New Foundland, Canada), CHARLES BURNHAM (University of Minnesota), STEPHAN CHAPMAN (Clemson University, South Carolina) DOUGLAS DEWEY (USDA, SEA, Utah State University), FRIEDRICH EHRENDORFER (University of Vienna, Austria), FRED ELLIOTT (Michigan State University), STEVE FRANSEN (South Dakota State University), WERNER GOTTSCHALK (University of Bonn, Fed Rep of Germany), WAYNE HANNA (Georgia Costal Plain Experiment Station), M L H KAUL

(University of Bonn, Fed Rep of Germany), ALEXANDER MICKE (International Atomic Energy Agency, Vienna, Austria), ROSALIND MORRIS (University of Nebraska), ROBERT NILAN (Washington State University), PHILLIP PALLISTER (Shodair Hospital, Helena, Montana), RALPH RILEY (Plant Breeding Institute, Cambridge, Great Britain), OWEN ROGERS (University of New Hampshire), VAL SAPRA (Alabama A and M University), ERNEST SEARS (USDA, SEA, University of Missouri), ROBERT SHERWOOD (USDA, SEA, Pennsylvania State University), ROBERT SOOST (University of California, Riverside) G LEDYARD STEBBINS, Jr (University of California, Davis) J SYBENGA (Agricultural University, Wageningen, Netherlands) TAKUMI TSUCHIYA (Colorado State University), BRUCE YOUNG (USDA, SEA, Grassland Forage Research Center Temple, Texas), CHENG YU (Lousiana State University Medical Center) DAVID CAMERON, HELEN CAMERON, THOMAS CARROLL, SCOTT COOPER, EUGENE HOCKETT, ERHARD HEHN, HOMER METCALF, PIERCE MULLEN, JOHN RUMELY, DAVID SKAAR, ERNEST VYSE, and GUYLYN WARREN (Montana State University) I appreciate the efforts of all these scientists, many of whose suggestions have been incorporated into the text However, I am aware that continued improvement is always possible and I will therefore, be grateful for further corrections and suggestions

I also thank all others who helped with the typing, proofreading, reviewing and indexing Finally, I thank my loving family, RUTH, IRIS ROSE, HEIDI, and CHRISTINA, who, through their patience and help, have made this book possible

August, 1980 JURGEN SCHULZ-SCHAEFFER

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Part I Introduction

Chapter 1
History of Cytogenetics*

Many reviews of the history of biology have been written Different approaches have been used in writing these reviews This is not just another list of dates to be added to the multitude that has already been published Instead, the author presents some of the people who have contributed to the advancement of a science, the motives that led them to choose their subject of investigation the reasons for choosing those subjects and the prior advances that made their contributions possible Cytogenetics was developed from two originally separate sciences—cytology and genetics Cytogenetics deals with the study of heredity through the methods of cytology and genetics The science is concerned with the structure number, function and movement of chromosomes and the numerous variations of these properties as they relate to the transmission recombination and expression of the genes It also deals with nonchromosomal hereditary factors

To fully understand the history of cytogenetics one has to look at its roots Consequently this history of cytogenetics includes the histories of cytology and genetics as well as cytogenetics The men who were chosen to be featured in this historical sketch made significant contributions to these sciences and, in this sense, represent milestones Many other important contributions were made by other men, all of whom could not be mentioned here

Johannes Sachariassen and **Zacharias** (1588–1631) **Janssen**, two Dutch eyeglass makers, father and son, between the years 1591 and 1608 produced the first operational compound microscope They combined two double convex lenses in a tube The magnification was not more than ten times, but it nevertheless caused great excitement

William Harvey (1578–1657) in 1651 put forward the concept that all living things including man originate from eggs and that the semen has a vitalizing role in the reproduction process Harvey was a court physician to King Charles I of England later he became a professor at Oxford While he was the king's physician, though he once dissected a doe in the King's forests, his privilege as the King's physician He found a fetus in the uterus of the doe This initiated his interest in the conception of life He eventually dissected more than 80 different species of animals

*Altered and reprinted with permission of the publisher from Schulz Schaeffer, J , 1976 A Short History of Cytogenetics Biol Zentralbl 95 193–221

Since he did not possess a microscope he was naturally unable to observe the eggs of mammals, but he presupposed their existence on theoretical grounds a conclusion that was confirmed long afterward Harvey also developed the *theory of epigenesis* which states that in the course of embryonic development new structures and organisms develop from an originally undifferentiated mass of living material

Marcello Malpighi (1628 1694) a professor at Bologna, Italy and later private physician to the Pope discovered the microscopic anatomy of both animals and plants In 1661 he discovered the capillaries in the lungs of animals and, thus, completed the story of the circulation of the blood as told by Harvey He was interested in the development of plant seeds the structure of plant stems and roots and the function of leaves as well as in the anatomy of the silkworm, the development of the chick embryo and the microscopic structure of glands and tissues

Robert Hooke (1635–1703) an architect as well as a microscopist and the first curator of the Royal Society of London in 1665 described cork and other cells and introduced the term cell His was the first drawing ever made of cells Microscopes at that time magnified 100 to 200 times with a distortion of shape and color that increased with magnification Nevertheless these microscopes revealed many new things Still it was necessary to wait for better lenses to see anything more Scientists waited for 160 years, and during this period they naturally argued about what they had seen

Regnier de Graaf (1641 1673) a young surgeon from Delft, Netherlands in 1672 discovered follicles in the human ovary and identified them, incorrectly as eggs They were named after him—**Graafian follicles** He discovered these follicles after he observed that the progeny of mammals presents characteristics of both the mother and the father therefore he reasoned, both sexes must transmit agents of heredity In search of some physical evidence for this observation, he studied sections of ovaries prepared for examination He found that before conception there were small watery lumps on the surface of the ovaries He observed them first in rabbits then in ewes and finally in human beings Although Graaf did not see the eggs it now seemed certain that female mammals and women produced eggs like the ones laid by birds or fishes It also seemed certain that the egg contained within itself the role and universal principle of heredity for all life Those who supported this view became known as ovists

Nehemiah Grew (1628–1711) an English physician and plant anatomist, worked with Hooke in London and described bladders and pores in wood and pith Grew was an ardent student of plant structure He published two illustrated volumes on the microscopic anatomy of plants (1672 1682) In these volumes he advanced a theory that the pistil in plants corresponds to the female and the stamen with its pollen to the male These were the first consistent studies leading to an understanding of the reproductive parts of plants In all he published well over one hundred engravings made from drawings of what he viewed through his microscope

Pierre Louis Moreau de Maupertuis (1698–1759) President of King Frederick the Great's Academy of Sciences in Berlin and a member of the Royal Society of London described an autosomal dominant pattern of inheritance in the polydactyly of men and discussed a concept of segregation His study of human pedigrees was a novel enterprise at that time He described this enterprise no less than three different times (1745 1751 and 1752) His studies were also the basis upon which he founded his theory of the formation of the foetus and the nature of heredity a theory that brilliantly anticipated the discoveries of Mendel and de Vries He applied the mathematical theory of probability to genetics a century before Mendel and undertook experiments in animal breeding to throw light on his theories

Joseph Gottlieb Kölreuter (1733–1806) German botanist during 1761–1766 published information about hybrids between plant varieties that might resemble one parent or the other or present a combination of their features Camerarius was the first to experiment in this field For a number of years Kölreuter crossed different types of tobacco with one another Later he crossed other plant genera such as pinks *Aquilegia Verbascum* and others One of his most valuable observations on reciprocal crosses showed the equality of contributions from the two parents Thus he provided clear evidence that in reciprocal crosses the hereditary contribution of the two parents to their offspring was equal

Jean Baptiste Pierre Antoine de Monet, better known to history as the Chevalier de Lamarck (1744–1829) was a discharged lieutenant who at the age of fifty was made professor of zoology in Paris France and attained lasting fame despite his lack of formal scientific training In 1809 he theorized that species can change gradually into new ones through a constant strengthening and perfecting of adaptive characteristics and that these **acquired characteristics** are transmitted to the offspring This theory is often called Lamarckism He also stated the importance of the cell in the living organism

Karl Ernst von Baer (1792–1876) a professor at Königsberg Germany discovered the mammalian egg and published two famous embryological works (1827–1828) He found that there are microscopic specks of jelly inside the Graafian follicles on the surface of the ovary of the rabbit. He had also found that there are similar specks in the oviduct entering the womb He had rightly concluded that these specks one eightieth of a centimeter across which happen to be the largest cells in the body were the female germ cells They were the eggs long ago imagined by Graaf But it was not before 1854 that a sperm was seen making its way into the egg of a frog and it was not until the following year that the same process was seen in various weeds and algae

Robert Brown (1773–1858) a Scottish botanist in 1828 discovered the cell nucleus in the flowering plant *Tradescantia* Although he practiced medicine as a surgeon for five years he later abandoned this and turned his efforts toward botanical sciences He was librarian to the Linnaean Society and curator at the British

Museum His remarkable account (1828) of the properties and behavior of the nucleus stand unmodified and without correction He was a very skillful and careful observer He also observed the random thermal motion of small particles still known as **Brownian movement**

Hugo von Mohl (1805 1872) a German medical doctor and professor of physiology and later of botany is generally mentioned as the creator of modern plant cytology In 1835 in his doctoral dissertation he described cell division and emphasized the importance of protoplasm He upheld clearly and convincingly that the cells in algae and even higher plants arise through partition walls being formed between previously existing cells These partition walls he investigated and described with great accuracy

Matthias Jacob Schleiden (1804 1881) is credited together with Schwann with proposing the **cell theory**, which states that the cell is a unit of biological organization Schleiden studied law and took up practice as an advocate Later he became a professor of botany at Jena Germany He became famous with the publication of his *Contributions to Phytogenesis* in 1838 He recognized the importance of Brown s discovery of the cell nucleus which Brown himself had failed to do and sought to reconstruct the course of development of the cell for which he wisely chose the embryonic cell as the starting point of his study He also discovered in cells *the formation of* what is now known as the ***nucleolus***

Theodor Schwann (1810–1882) a German professor of anatomy began work with Schleiden s cell formation theory which he accepted in its entirety and expanded it into a general theory of the basis and origin of all life phenomena He applied Schleiden s discoveries in plants to animals and invented the term **cell theory** in 1838 however the term is generally attributed to Schleiden and Schwann and dated 1839 Schwann refined this theory in the following way

1 The cell is the smallest building element of a mult cellular organism and as a unit is itself an elementary organism
2 Each cell n a multicellular organism has a specific task to accomplish and represents a working unit
3 A cell can only be produced from another cell by cell division

This concept of the cell as a general unit of life and as a common basis for the vital phenomena in both the animal and vegetable kingdoms was immediately and universally accepted

Rudolf Ludwig Carl Virchow (1821–1902) a German professor of pathological anatomy was a figure of great importance in the intellectual social and political history of nineteenth century Europe He confirmed the principle that cells arise only from preexisting cells which is also referred to as the **theory of cell lineage** Diseases and their causes were the chief objects of Virchow s studies and this led him to the realization of the cells as basic constituents of the organism both in health and sickness In 1858 he published his *Cellular Pathology* a theory identifying the cells as the true causes of disease His observations gave Schleiden and

Schwann s cell theory its impact in terms of heredity and development for if pres ent cells have come from preexisting cells then all cells trace their ancestry back to the first cells in an unbroken line of descent

Gregor Mendel (1822 1884) prelate of an Austrian monastery developed the fun damental principles of heredity In 1866 he published his famous paper on *Exper iments in Plant Hybridi ation* describing his garden pea experiments This paper was the first to come from many years work, and it testified to a keen observation of nature and a thorough grounding in mathematical thought His findings were obscured for many years but finally were rediscovered in 1900

Peas have many advantages for research They are easy to pollinate and to protect from foreign pollen Mendel noted the points of resemblance and difference between certain varieties and convinced himself of the constancy of several pairs of characteristics such as the round or wrinkled shape of the seed its yellow or green color the different colors of the seed pods and the tallness or dwarfness of the plants He then studied these characteristics through several daughter genera tions of the hybrids He did not merely note the development of the characteristics or their failure to appear in the hybrids but determined the frequency of their appearance in the progeny resulting from various crosses His counts put the phe nomena of inheritance for the first time on a numerical basis and the new princi ples which he eventually revealed became known as Mendel s Laws which are

1 **Law of Segregation** There are pairs of factors within the sexual organism and one factor of each pair goes into each mature germ cell Therefore each member of a pair segregates from the other in the parent and reunites in the offspring This law deals with one pair of genes and discusses the behavior of genes or alleles at the same locus
2 **Law of Independent Assortment** Each of the genes segregates from the other and pairs again in an independent fashion thus giving rise to new combinations of characteristics This law deals with two or more pairs of genes and discusses the behavior of genes or non alleles at separate loci

Francis Galton (1822 1911) an English explorer and Fellow of the Royal Society in 1869 published *Hereditary Genius* and so founded the scientific study of human heredity This book also introduced the statistical method into the study of heredity In 1876 he reported on studies of human twins in which he tried to separate the effect of heredity and environment He developed the concept of regression, a measurement of the degree of resemblance of relatives He also developed the concept of quantitative analysis of continuously variable or polygenic traits such as diabetes and height

To the science that he placed highest of all he gave the name eugenics, a name that has become universally accepted According to Stern (1960) eugenics is the study of agencies under social control that may improve or impair the hereditary physical or mental qualities of future generations of men

Fredrick Miescher (1844–1895) a Swiss chemist, in 1871 reported that he had isolated nucleic acid and nucleoprotein By that time it was clear from its universal occurrence that the nucleus was a peculiarly important cell constituent However before any chemical analysis of the nucleus was possible it was first necessary to

separate, in quantity nuclei from the remainder of the cell Miescher decided to attempt this and made, what seems at first sight, the bizarre choice of pus cells He found that it was possible to get the cells in suspension and furthermore, by treatment with dilute hydrochloric acid, pepsin, and ether, to separate the nuclei from everthing else From those nuclei he prepared a substance of remarkable properties, namely nucleic acid, which later became known as DNA It was distinguished by a high content of phosphorus, an element then rarely found in organic substances of physiological origin So remarkable did Miescher's results then appear that the publisher Hoppe-Seyler was reluctant to print them until he had himself confirmed Miescher's conclusion by experiment

Then Miescher worked on Rhine winter salmon sperm In the isolated heads of the sperm he not only found nucleic acid but also a highly basic nitrogenous substance to which he gave the name protamine When protamine is combined with nucleic acid, the compound is referred to as nucleoprotein

Wilhelm August Oscar Hertwig (1849–1922) a professor of anatomy, in 1876 and 1877 studied reproduction in the sea urchin, *Paracentrotus lividus* and concluded that fertilization involves the union of sperm and egg This study initiated the period of experimental cytology

When he was younger Oscar, along with his brother Richard, was supposed to take over their father's factory, but a high school teacher recognized their natural scientific abilities and prevailed upon their father to allow his boys to study chemistry At the University, however, the professor of chemistry turned out to be so dull that the brothers soon changed to medicine, which also included zoology

When he became a professor of anatomy, Oscar Hertwig chose the eggs of sea urchins for his first investigations of reproduction This proved to be a good choice because many favorable conditions are met in the sea urchin, which is still unsurpassed today for many experimental purposes It is particularly favorable for observations of living cells The discovery of a new favorable object of investigation often opens an entire new area of research The eggs of the sea urchin are transparent, small (0 1 mm in diameter) and favorable for studies under high magnification Hertwig was able to watch the sperm nucleus pass through the translucent cytoplasm of the egg and realized that it would unite with the ovum nucleus Until Hertwig's investigations only unfavorable objects like the nontransparent eggs of frogs were used, eggs that were fertilized inside the mother and could not be observed easily

Walter Flemming (1843 1915), an Austrian cytologist, in 1882 proposed the term *mitosis* He showed that the chromosomes split longitudinally during nuclear division and the formation of daughter nuclei He also applied the name **chromatin** to the stainable portion of the nucleus He was a distinguished observer, technician and teacher

In an important paper (1879) he described mitosis in living and fixed cells of the salamander An essential contribution was his development of improved fixing and staining methods to make visible cytological details His monograph (1879)

included a remarkably foresighted treatment of the problems of cell division, some of which are still active research problems today In 1882 the studies on the human chromosome complement began with Flemming's demonstration of cell division in the corneal epithelium of humans

August Weismann (1834–1914), a German biologist, in his essays of 1883 and 1885 put forth his **germplasm theory,** which was an alternative explanation to Lamarck's theory of acquired characteristics Weismann s theory was based on the early separation, in the animal embryo, of the germplasm from the somatoplasm It emphasized the remarkable stability of the hereditary material Conceivably, little if any, environmental influence could affect the genes, even though environmental modifications of external characteristics occurred Reproduction in animals was accomplished not by body cells or somatoplasm but by the germplasm, which was transmitted essentially unchanged from generation to generation
The fundamental premise is well established, although some details of the germplasm theory have been modified Weismann speculated that the chromosomes of the sex cells were the carriers of his germplasm, but he erred in assuming that each chromosome could contain all hereditary material He also postulated that a periodic reduction in chromosome number must occur in all sexual organisms and that during fertilization a new combination of chromosomes and hereditary factors takes place His theory was that the alternation of reduction and fertilization is necessary for maintaining constant chromosome numbers for sexual reproduction At that time this process had not been observed under the microscrope, and its mechanism was a matter of speculation

Wilhelm Roux (1850–1924), a German zoologist, in 1883 proposed that it was the chromosomes that contain the units of heredity He speculated on the question of how the hereditary units could behave in such a way that each daughter cell receives all that is in the parent cell and becomes a complete cell and not half a cell or only part of a parent cell
The only mechanism he could devise to test his speculation was to line up objects in a row and duplicate them exactly He therefore suggested that the significance of cell division lies in the fact that nuclei have strings of bead like structures that line up and duplicate themselves If nuclei really have such structures, he reasoned, it might be possible to explain the mechanics of hereditary transmission from cell to cell The most likely constituents of the nucleus to fill these requirements were the chromosomes His hypothesis was that not only the chromosomes but individual parts of each chromosome were important in determining the individual's development, physiology, and morphology Proof of this hypothesis was not given until later This was in direct contrast to Weismann's idea, that each chromosome could contain all hereditary material

Edouard van Beneden (1845–1910), a highly reputed Belgian zoologist, in 1883 showed that in the round worm, *Ascaris megalocephala* the number of chromosomes in the gametes is half the number that is in the body cells, and that in

fertilization, the chromosome contributions of egg and sperm to the zygote are numerically equal Through this observation he confirmed Weismann's theory on reduction and fertilization

Ascaris was a favorable object for his studies This species has only $2n=8$ chromosomes and in a particularly favorable race only $2n=4$ Also the size of its chromosomes make them ideal for observation Basic to the clarification of the role of the chromosomes as the physical agents of Mendelian phenomena was the discovery of their behavior in meiosis In his monograph, Beneden (1883) traced the spermatogenesis and oogenesis of the round worm and proved Weismann's theory of reduction and fertilization Confirmation of these discoveries soon followed, but the exact mechanism of reduction by synapsis and the formation of bivalent pairs of chromosomes was not understood until the 1890s

Carl Wilhelm von Nägeli (1817–1891), a Swiss botanist, though certainly best known as a cytologist, in 1884 made his major contribution with his theory of the **idioplasm** According to this theory, the idioplasm is the sum of all hereditary determinants of an organism, which in its fine structure ought to contain the **life formula** of that organism With this theory he provided the chief stimulus for the view that there is a single hereditary substance This theory led Beneden, Strasburger Oscar Hertwig and Weismann to the belief that the idioplasm is located in the substance of the chromosome Thus, nuclear division and chromosomes were shifted into the center of a uniform and large field of biological studies In 1842 Nägeli published the first drawings of chromosomes

Eduard Strasburger (1844–1912), a German cytologist, in 1884 described fertilization in angiosperms In the field of plant cytology, he was preeminent in his time Equally distinguished as a research worker and a teacher, he attracted a large number of students from many countries to his institute He was a leading writer of textbooks, and his scientific productivity included his epoch making cell studies (1884, 1904, 1905) Strasburger demonstrated that the principles of fertilization developed by Oscar Hertwig for animals held also for plants

Hertwig and Strasburger are considered the discoverers of fertilization Before Mendel's work was discovered, interest had already developed in locating the source of hereditary transmission Since sex cells, that is, eggs and sperm were known to be involved in fertilization and both parents were known to transmit their characteristics to the progeny, the first problem was to determine which part of the cell was involved in hereditary transmission Strasburger observed that the egg carried more cytoplasm than the sperm Just like Kölreuter 100 years before him Strasburger made reciprocal crosses between different plant species and found that the results were similar Since the egg and sperm were unequal with respect to size and amount of cytoplasm carried, he suggested that the cytoplasm was not responsible for hereditary differences between species Consequently, he came to the conclusion that the nucleus and its chromosomes are the material basis of heredity and at the same time, the material governing development Strasburger stated that molecular stimuli are passed from the nucleus to the cytoplasm that surrounds it,

controlling the process of metabolism in the cell and giving a specific characteristic to its growth

It is an important historical fact that after the German zoologist Otto Bütschli had discovered and understood mitotic division, the botanist, Strasburger called on him, studied his preparations, and realized that what he had seen in plant cells was exactly the same thing Since then cytology has not made a serious distinction between animals and plants All the basic facts of chromosomal structure and behavior mitosis fertilization, sex chromosomes cytoplasmic inclusions, and cell physiology are identical Thus, cytology developed into an independent science drawing its discoveries from animals, plants and humans

Ernst Abbe (1840–1908), a German physicist, by 1886 had produced oil immersion objectives with a resolution of 0 25 μm This advanced the resolving power of the light microscope to the absolute limit set by the wave length of light A further advantage of this system was that the performance of these lenses was independent of the thickness of the coverslip Abbe was head of the German Zeiss corporation which was the leading microscope manufacturer

Early in the 1880's Abbe joined with Otto Schott, a glass manufacturer, in experiments on adding various chemical elements such as boron and phosphorus to the silicate base of glass By 1886 they had produced their Jena glass, which had novel characteristics The improved lenses that these new materials made possible were called **apochromatic,** because they eliminated the residual chromatic aberration, the secondary spectrum, of the achromat Cytologists such as Hertwig and Flemming were using apochromatic objectives within a few years of their introduction

Theodor Boveri (1862–1915), a professor at Würzburg, Germany, and a student of the brothers Hertwig in his celebrated *Zellstudien* (1887, 1888) together with Oscar Hertwig (1890) discovered the real nature of reduction division In 1892 he described meiosis and, particularly, synapsis in *Ascaris* He also explored the question of the source of hereditary transmission in animals, which Strasburger had studied in plants

By shaking sea urchin eggs at a critical time in their development, he produced some eggs without nuclei and some with nuclei as usual Each of these kinds of eggs were fertilized by a normal sperm from another species of sea urchin Eggs lacking a nucleus produced larvae resembling the species from which the sperms were obtained, but those with nuclei developed into hybrids, showing the characteristics of both species The cytoplasm in the two kinds of eggs had not been altered and it was therefore presumed that the nucleus and not the cytoplasm was responsible for the transmission of hereditary traits

With his experiments on the double fertilization of sea urchin eggs, *Toxopneustes* (1902, 1904, 1907), Boveri also contributed to the formulation of the **chromosome theory of inheritance,** which will be discussed later He found eggs that had been fertilized by two spermatozoa Since each sperm introduced a centrosome into the egg, and each centrosome divided in anticipation of the first cleavage division, the initial metaphases and anaphases were often characterized by a **tetraster,** which is a spindle with four poles Since the dividing nucleus was triploid, the distribution

of the chromosomes to four poles in anaphase was irregular Boveri isolated many of the first division blastomeres from these dispermic eggs and demonstrated that most were abnormal in development but that all were not alike in their abnormalities He concluded that abnormal development resulted from the irregular distribution of chromosomes brought on by the multipolar division Each chromosome must consequently have possessed a certain individual quality that expressed itself in development

Hermann Henking (1858 1942) a German zoologist in 1891 described in the hemipteran insect *Pyrrhocoris* chromatin elements that he labeled *X* and that now are known to be the sex or X chromosomes He found a peculiar chromatin element that in the second spermatocyte division first lagged behind the separating anaphase chromosomes and then passed undivided to one pole while all the other eleven chromosomes were equally divided From this it followed that the sperms were of two numerically equal classes distinguished by the presence or absence of this chromosome element This element had to have a close relationship for the determination of sex If the egg was fertilized by one class a male was formed if by the other a female All the essential features of Henking s description were subsequently confirmed in other animals by other observers This mechanism is now called the XO system of sex determination

Edmund Beecher Wilson (1856 1939) an American biologist and Professor at Columbia University was a superb synthesist as well as a stimulating teacher and investigator By 1896 he had been able to organize the cytological and embryological knowledge of his day in the first edition of his classic *The Cell in Development and Inheritance* Mendel s principles of genetics were still to be rediscovered but the beginning of cytogenetics and of the **chromosome theory of inheritance** were clearly outlined by Wilson s statement that the visible chromomeres on the chromosomes were in all probability much larger than the **ultimate dividing units** and that these units must be capable of assimilation growth and division without loss of their specific characteristics Wilson brought the past in relation to the future Four principles were laid down by Wilson as the foundation of the chromosome theory

1 The exact lengthwise division of the chromosomes at mitosis allows for the equal distribution of linearly arranged particles to the daughter cells
2 The assumed material existence of the chromosomes in the nucleus between mitoses gives the genetic continuity necessary for the organs of heredity
3 The fact that the nucleus goes where things are happening shows its governing position in the work of the cell
4 The equality of the chromosomes of the fusing germ cells corresponds to the equality of male and female in heredity

These arguments had long been known but were still widely disputed or misunderstood at this time

Carl Franz Joseph Correns (1864–1933) a German botanist in 1900 along with Hugo de Vries and Erich von Tschermak was one of the three rediscoverers of the fundamental principles of heredity first developed by Mendel in 1866 He had

carried out extensive hybridization experiments on maize stocks *(Matthiola)* beans peas and lilies at the University of Tübingen during the 1890s In 1899 he had data from several generations of garden pea and maize and had arrived at conclusions similar to those of Mendel He studied Mendel s paper because he had read a statement that Mendel believed he had found constant numerical relationships in his experiments Correns compared his own and Mendel s data and in 1900 he reported that he had observed the same kind of results with maize He disagreed with de Vries in that he thought there were cases that did not con form to the Mendelian scheme

Hugo de Vries (1848 1935) a Dutch biologist and rediscoverer of Mendel s laws was also known for his mutation theory and studies on the evening primrose and maize De Vries published three papers on Mendelism in 1900 one of which for the most part has been overlooked He later stated that he had worked out the Mendelian scheme for himself and was later led to Mendel s paper
In the 1880s de Vries a keen observer and objective scientist saw striking vari ations in the plant called Lamarck s evening primrose *Oenothera lamarckiana* which had been introduced from America and had grown wild in Europe He collected seeds from plants that differed from the standard type and raised them in his botanical garden at Hilversum a few miles east of Amsterdam On careful observation many differences in growth form were seen among the different plants One type of *Oenothera* called *gigas* was much larger than the average and no intermediate gradations were observed between it and smaller types It seemed to represent a distinct and discontinuous change from the usual size On one occasion a gigas plant was found alone in a bed of plants of the standard size The new plant produced only giants like itself A dwarf type called *nanella* which gave rise only to dwarfs was also observed Other abrupt changes that affected the color and shape of various parts of the plant were studied and the variations seemed to breed true
De Vries visualized these changes as a source of variation in evolution in contrast to the gradual process suggested by Lamarck and Darwin The word **mutation** implying change was used to describe such alterations In 1901 de Vries published his accumulated data in a book entitled *The Mutation Theory* Mutations were considered to be rare in nature but capable of providing variations by which races and species were distinguished De Vries was careful to make a distinction between hereditary and environmental variation but his mutations are now known to include changes in chromosome structure and number The term mutation today is used in a more restricted sense to specify only gene changes or point mutations and not visible chromosome changes

Erich von Tschermak (1871 1962) an Austrian botanist is also considered to be one of the rediscoverers of Mendel s laws His interests were in practical plant breeding and this led to studies of the effects of crossing and inbreeding on vege tative vigor in peas He published two papers on the subject in 1900 He later wrote that the three rediscoverers were fully aware of the fact that the independent dis covery of the laws of heredity was far from being the accomplishment it had been

He can be closely identified with the development of genetics as a science although his early scientific work was in the field of plant physiology. His first genetic paper *On Heredity and Variation*, appeared in 1896 and in 1898 he began the investigations that have since become classics on barley and beans.
In his paper on *Pure Lines* (1903) he showed a difference in the effects of selection when applied to populations of ordinary cross fertilizing organisms as compared with self fertilizing ones. Self fertilization was found to produce homozygosity or pure lines. In cross breeding populations, selection was found to be effective in altering the proportion of different types. When plants were self fertilized over long periods selection was no longer effective. The plants had become completely homozygous and no genetic variation was left for selection to act on. All variation in a pure line is environmental.

Frans Alfons Janssens' (1863–1924) name is generally associated with the partial chiasmatype theory, which he advanced in 1909. In 1905 he described the configurations of the bivalent pairs in the spermatogenesis of Amphibia that showed chiasma like configurations. He indicated that chiasmata are produced by exchange between chromatids of nonhomologous chromosomes and later suggested the possible genetic significance of these crossed segments of the chromosomes. The partial chiasma type theory postulates that true chiasmata are the direct result of crossing-over, being formed at precisely the points where the exchange of segments between non sister chromatids took place. This theory is now the most satisfactory account of the relationship between the cytologically visible chiasmata and genetic crossing over.

Thomas Hunt Morgan (1866–1945) in 1910 discovered the mutant *white eye* and consequently sex linkage in *Drosophila*. With this discovery *Drosophila* genetics had its beginning.
Drosophila melanogaster the fruit fly, proved to be one of the most ideal laboratory animals for cytogenetic studies. It is a tiny organism, about 6 mm long. Completing its life cycle from egg to fly in about 10 days, this insect supplies as many as thirty generations a year, an enormous advantage compared to the relative slowness of the usual laboratory animals. It is easily bred, fertile, and with a life span that can reach ninety days. Thousands of these flies can be handled in a few milk bottles, while the cost of feeding and upkeep is negligible. The giant chromosomes in the salivary glands are several hundred times larger than normal somatic chromosomes, and the bands reveal the necessary detail for cytogenetic study. The low chromosome number of $n = 4$ also is ideal.
Within a short time, Morgan's fly room at Columbia University in New York became a very popular place. A steady stream of American and foreign students, both doctoral and post-doctoral, passed through his laboratory. Morgan was concerned about the exceptions to Mendel's second law of independent assortment. This law implies that an organism cannot possess more gene pairs than the number of chromosomes in a haploid set, if it is granted that the genes are borne on chromosomes. Within the first decade after the rediscovery of Mendelism, this logical consequence of the theory was sharply contradicted by experience.

Obviously some extension or revision of the theory was necessary Morgan s alternative linkage theory supposed that genes are organized in a definite linear order within the chromosome These genes are expected to exhibit linkage if they lie within the same chromosome but should they lie in nonhomologous chromosomes they would be transmitted according to the principle of independent assortment The possibilities of recombination for linked genes were thus envisioned to depend on the breakage of chromosomes and their rejoining in such a way as to result in the exchange of equal segments without disturbance of the basic linear sequence It must be recalled at this point that Morgan s theory unlike the purely formalistic approach of Bateson and Punnet (1906) rested solidly on a body of accumulated cytological evidence concerning the intimate details of chromosome behavior dur ing the prophase of the first meiotic divisions The first test of the validity of these assumptions was provided by Morgan in 1911 when he showed that several sex linked mutants in *Drosophila* were associated with the behavior of the heteromorphic sex chromosomes During the following decades thousands of experiments in a wealth of diverse biological forms have confirmed the universality of Morgan s interpretation of linkage In 1933 Morgan was the first to receive the Nobel Prize in medicine and physiology for accomplishments in the field of genetics for his development of the theory of the gene

Ralph A Emerson (1873 1947) together with E M East in 1913 published a paper on maize in which they reported that the F_2 was much more variable than the F They interpreted this as being due to the segregation of several pairs of genes Their joint paper is a class c in the field of genetics and marks the bringing of the **inheritance of quantitative characters** into the general scheme of Mendelism In 1914 Emerson discovered the first mutant in maize *blotched leaf (bl* Chrom 2 Emerson et al 1935)

Emerson the son of a long line of American farmers including the celebrated Adams family was teaching horticulture at the University of Nebraska Later he came to Cornell University where he trained a group of workers in genetics who are now spread all over America and initiated a remarkable organization for cooperative work The maize work which started independently of the *Drosophila* work took fresh impetus from the publication of the first important *Drosophila* papers The disadvantage of maize was that as mentioned before the fruit fly would produce 30 generations before the maize plant completes one But the maize workers had one great advantage over the fly workers the chromosomes in meiotic maize cells are more easily studied under the microscope and this cytological simplicity made their mapping less difficult Most of the delicate cytogenetic work in maize was later done by Barbara McClintock, one of the most skillful and persevering of the younger genetics workers in America at that time

In 1914 Emerson also suggested that some genes might not be completely stable He studied a variegated variety of maize that had a white pericarp with numerous red spots of varying size Genetically these plants were homozygous for the recessive gene for *white* Emerson concluded that this gene must be unstable that it can mutate spontaneously into the dominant allele for *red* and that each red spot on the kernel is made up of cells that came from one cell in which such a mutation

arose This was a very revolutionary idea at that time Such unstable genes are now referred to as **mutable genes.**

Albert Francis Blakeslee (1874 1954) American botanist and geneticist, in 1921 discovered **trisomics** in the Jimson weed, *Datura stramonium* a plant species from which the drug belladonna is obtained He worked at the Cold Spring Harbor Station for Experimental Evolution for the Carnegie Institution of Washington In 1937, together with Oswald T Avery he discovered that doubling of the chromosome set in plants may be induced by use of the alkaloid colchicine After Blakeslee discovered trisomics, he initiated the first critical study of aneuploid plants with Belling (1924) The trisomics differed morphologically from the wild-type plants in several specific ways Conspicuous deviations from the normal were observed in the shape and spine characteristics of the seed capsules These traits were associated with the extra chromosome which gave the plant an extra dose of all those genes contained in the extra chromosome It was thus possible to identify some genes with their chromosomes giving further evidence that certain genes are located in particular chromosomes

Calvin Blackman Bridges (1889–1938) a research associate of the Carnegie Institution of Washington, in 1923 was the first to discover **duplications, deficiencies,** and **translocations** in *Drosophila* chromosomes He also observed triploid intersexes in *Drosophila* Bridges joined T S Painter's investigations of the giant salivary gland chromosomes for further refinement of technique and fuller and more salient details He stretched the chromosomes of the salivary gland cells until they were more than 150 times longer than those of the egg cells He made preparations from larvae that had been raised to their maximum size by supplying them with an extra diet of yeast
Bridges kept working, studying deficiencies and duplications, in an effort to revamp his chromosome maps In 1935 he published the first complete map of all four *Drosophila melanogaster* chromosomes Several revised maps of these chromosomes were published later The genetic linkage map was superimposed on the cytological map He also devised a special numbering system that is still used today to identify particular bands illustrated In 1936 his son, Philip N Bridges, came to his help Calvin Bridges, a brilliant, simple, and unaffected worker, never finished this study He had driven himself so hard during this work that he died of a heart attack in Los Angeles in 1938

Robert Joachim Feulgen (1884–1955), German biochemist, in 1924 together with H Rossenbeck described a test for the presence of DNA This specific staining reaction is now called **Feulgen reaction.** Through this reaction it was proven that DNA is located in the chromosomes of interphase cells In 1914 Feulgen showed that the unstable carbohydrate of the thymus type of nucleic acid was not a hexose, as it had been regarded until then, but a pentose On gentle hydrolysis this pentose liberated aldehyde, which could be detected by the usual reagent for this class of substance, the dye fuchsin decolorised by sulphurous acid Ten years later this test was applied to sections of tissue under the microscope Feulgen and Rossenbeck

were then greatly surprised to find that the nuclei of the wheat germ gave a strong reaction to this test, for this result showed that a nucleic acid of the thymus type could be found in plant cells The thymus type of nucleic acid, of course, is now known as DNA The full significance and potentiality of the Feulgen reaction, applied as histochemical method, only slowly became understood Cytologists did not begin to employ it until the late 1920s This method is still the safest one to distinguish DNA or chromatin from cytoplasm and nucleoli or RNA

Herman Joseph Muller (1890–1967) in 1912 joined Morgan's fly group at Columbia University with an assistantship in zoology In 1915 he completed his Ph D study with a thesis on fruit flies called *The Mechanics of Crossing Over*, showing how genes are exchanged between chromosomes Columbia University was an exciting place during Muller's graduate years, the young science of genetics was getting greater impetus in America

In 1914 while working for his doctorate, he came across a new fly with a bent wing The usual routine for a new mutant was followed in an attempt to find its linkage group After an elaborate series of selected breeding experiments, the new character refused to associate itself with any of those in the three demonstrated linkage groups of chromosomes The obvious conclusion was that it belonged to the small fourth chromosome that all this while had been floating apparently uselessly in the nucleus waiting for a mutant character with which to be associated What for a moment had appeared as an obstacle to the acceptance of the validity of the linkage theory was converted into additional evidence of its plausibility

The discovery of crossing over and the elucidation of its cytological basis necessarily occasioned a major revision of the gene concept that was to include another fundamental property In crossing over, genes behaved as units between which, but not through which exchanges occurred Once a mutant was detected it was a relatively simple matter to discover both the chromosome with which it was associated as well as its specific genetic or cytological locus on that chromosome But the study of gene mutation was seriously hampered by the low rate of spontaneous mutations This serious limitation to direct study of the gene was removed by the epochal discovery of Muller in 1927 that the mutation rate could be increased several thousand percent through the action of x rays For this discovery Muller received the Nobel Prize in 1946 In 1928, one year after Muller's paper, L J Stadler verified the increase of the mutation rate by x rays in plants

Hitoshi Kihara (b 1893), former Japanese Professor at Kyoto University, in 1930 formulated a method he called **genome analysis.** This method is designed to determine the diploid ancestors of allopolyploid species Several years earlier Kihara together with Ono in 1926 had introduced the terms **auto-** and **allopolyploidy** in order to better distinguish between these two important classes of ploidy The method of genome analysis was first used in such important plant genera as *Triticum Aegilops Nicotiana Raphanus Brassica* and *Rosa* The method consists of the analysis of meiotic chromosome pairing in the hybrids between polyploids and diploids If the diploid has at one time or another contributed to the formation of the polyploid, chromosome pairing should occur between two sets of homologous

chromosomes in the hybrid This method has subsequently contributed to the knowledge of systematic relationships between many cultivated and wild polyploid species

Curt Stern (b 1902) a German born American geneticist in 1931 presented cytological proof of crossing over in *Drosophila* This was done independently of McClintock s and Creighton s demonstration in maize during the same year Stern s study which involved the sex chromosomes provided considerably larger populations in which genetic crossing over was more precisely localized More specifically Stern used an X chromosome with an arm of a Y chromosome attached to its right end and an X IV translocation In both cases two marker genes between the cytologically identifiable regions were available and it was demonstrated that recombination between the marker genes was regularly accompanied by recombination between the cytological markers These papers gave the final cytological proof that genetic crossing over is accompanied by an exchange of parts between chromosomes Beyond any question these were some of the truly great experiments of modern biology

G k Chrustschoff et al in 1931 published a first attempt to study human chromosomes using cultures of leucocytes from peripheral blood In 1935 Chrustschoff and Berlin published details of culture techniques for human leucocytes This important paper has until recently passed relatively unnoticed and no effort was made at the time to adapt this technique to the chromosome analysis of humans

J Belling (1866–1933) in 1931 developed a new classical model of crossing over He studied plants of the lily and related families The cytological study of the meiotic processes was actively investigated at about this period to see what really happened at crossing over His model was based on the assumption of random breaking of the thin paired chromosome strands with reunion of the broken ends which could lead to interchanges between homologues if two breaks happened to occur at the same level He related the phenomenon to the production of new daughter chromatids an idea that has been involved in many of the more recent interpretations

Cyril Dean Darlington (b 1903) professor of botany at the University of Oxford in an attempt to explain meiosis advanced the **precocity theory** He published his opinions in a long series of papers and first developed the general scheme in *Recent Advances in Cytology* (1932) The scheme was very generally accepted and for a time was considered the very backbone of cytogenetics He assumed that the chromosomes have a tendency to be in a paired state at all times In mitosis this condition is met in that the chromosomes entering prophase are already double According to this theory meiotic prophase is assumed to start precociously with chromosomes that have not yet split and this is held responsible for chromosome pairing

Darlington said that the chromosomes are in an unsatisfied or unsaturated state electrostatically To become saturated they must pair homologously When the

chromosomes become double in late pachytene, the satisfied state is between sister chromatids instead of homologous chromosomes The paired homologues consequently fall apart and diplotene is initiated This theory was logically beautiful in superficially explaining the genetic implications of meiosis But since both DNA and protein synthesis have now been shown to be completed before meiotic chromosome pairing occurs, the precocity theory appears no longer to be valid
This theory is only one of the many thought provoking ideas that Darlington developed during this period These ideas were challenging and stimulating and initiated a wealth of research all over the world In 1929, for instance, Darlington coined the term **chiasma terminalization** in order to explain the progressive shift between diplotene and metaphase I in the distribution of chiasmata along the arms of paired chromosomes from their points of origin to more distal positions

Ernst August Friedrich Ruska (b 1906), Director of the Max Planck Institute for Electron Microscopy in Berlin, Germany, together with Knoll published a description of one of the first electron microscopes in 1932 Their instrument consisted of an electron source and two magnifying lenses A condenser lens was not used The resolution obtained with this instrument was below that attained with the light microscope Nevertheless, they obtained the first electron micrographs of an illuminated specimen
In 1934 Ruska described an improved version of this electron microscope to which a condenser lens was added The micrographs obtained indicated that the potential existed to surpass the resolving power of the light microscope The uniquely high resolving power of a microscope using electrons as the illuminating beam is explained quite simply from the fact that electrons have an associated wavelength smaller than that of any other radiation practicable for use in a light microscope system By 1940 commercial instruments with limiting resolving power of 2 5 nm were manufactured in Germany and America By 1946, improvement in technique and design made it possible to demonstrate resolutions from 0 85 nm to 1 5 nm Because the electromagnetic lenses that must be used for focusing the electron beam cannot be corrected for spherical and chromatic aberrations, the resolution limit of 0 8 nm to 1 nm is still the experimental and theoretical limit of the microscope, despite the approximate 0 005 nm wavelength of electrons
Most of the advances possible with the electron microscope have involved the cytoplasm of the cell since the chromosomes are notable for their lack of the membranous and granular structures so prominent in the cytoplasm Techniques of spreading interphase and metaphase chromosomes for electron microscopy are very recent developments that can contribute to new ideas of chromosome ultrastructure

Emil Heitz (b 1892), German geneticist, professor at the University of Tübingen and associated with the Max Planck Society in 1933 together with Bauer discovered the importance of the **giant chromosomes** in the salivary gland cells of dipteran insect species as important objects in cytogenetic research These structures had been discovered prior to this in 1881, but had not been identified as chromosomes They represent bundles of chromosome subunits or chromatids Since they are not spiralized they are about 100 to 150 times longer than ordinary mitotic

chromosomes This unusual length and their banding pattern make them very suitable for chromosome identification and gene localization
In 1928 and 1929 Heitz was the first to distinguish two types of chromatin which he named **euchromatin** and **heterochromatin.** Euchromatin stains lightly or not at all in interphase and prophase, while heterochromatin stains darkly in these stages Heterochromatin is an extremely helpful marker for chromosome mapping in the pachytene stage of meiotic prophase In 1931 Heitz showed a correlation between the number of nucleoli in the interphase nucleus and the number of a particular type of chromosome, now called the **nucleolus organizer chromosome** A study of these chromosomes indicated that the nucleolus is organized at a specific site on the chromosome

Tobjörn Oskar Caspersson (b 1910) head of the Medical Cell Research and Genetics Department of the Karolinska Institute of Stockholm Sweden, in 1936 began to develop ultraviolet photomicrography for the study of nucleic acids within the nucleus These substances absorb ultraviolet light very strongly in a most characteristic and selective fashion The method has the great advantage of being able to use unstained material as the object and, thus, the contrast in the resulting photomicrographs was indirectly due to the components themselves and not to their affinity for a stain, the depth of which is largely dependent on the conditions of use So the density of nucleic acids could readily be compared from one tissue to another It was found that wherever cells of tissues are growing rapidly, the density of nucleic acids within them was relatively high Evidence of this kind thus pointed to the conclusion that nucleic acids have some biological function in the process of synthesis within the cell It also helped to pinpoint the time period during which such nucleic acid synthesis does take place (Caspersson 1947)

George Wells Beadle (b 1903), later president of the University of Chicago, with Edward L Tatum in 1941 and in a monograph in 1945 developed the **one gene-one enzyme concept.** In 1958, together with Lederberg, they received the Nobel Prize in medicine and physiology for the discovery that genes regulate certain definite chemical processes Their study on the biochemical genetics of the pink bread mold, *Neurospora crassa,* is now a classic, and it marked a significant turning point in the analysis of the general problem of genetic control in metabolism and development
Instead of attempting to work out the chemical basis for known genetic characters, they deliberately reversed the procedure and set out to determine if and how the gene controlled known biochemical reactions The wild strain of bread mold could be made to grow on a medium containing sugar and inorganic salts The salts included nitrogen compounds out of which the mold was able to manufacture for itself all the necessary amino acids Not one amino acid had to be added to the medium Beadle and Tatum then subjected the spores of the mold to x-rays Occasionally, an irradiated spore would refuse to grow on the medium, but it would grow if a certain amino acid such as lysine were added Apparently, the irradiated spore had lost the capacity to manufacture its own lysine out of the inorganic nitro-

gen compounds. Without lysine it could not grow. If lysine were added to the medium it could grow. It seemed evident that an enzyme that normally would have catalyzed one of the reactions that led to lysine was not formed by the spore. Supposedly a particular gene had been mutated by the x rays.

According to their one gene-one enzyme hypothesis the gene controls the synthesis or the activity of but a single protein or enzyme with catalytic activity. Since its formulation this concept has been verified in principle even though it was controversial when first announced.

Oswald T. Avery (1877–1955) a member of the staff of the Rockefeller Institute Hospital, New York, together with MacLeod and McCarty in 1944 showed the significance of DNA as the hereditary material through studies of transformation in bacteria. The phenomenon that they called "transformation" involves a transfer of genetic information by means of naked extracellular DNA. They showed that purified DNA preparations extracted from a particular smooth strain of *Pneumococcus* bacteria can confer an inheritable smoothness on bacteria that were formerly rough. The experiments also showed that the preparations most active in bringing about transformation were those purest and most free of protein. This fact effectively cast doubt on the wide spread and commonly accepted belief that proteins were the mediators of biological specificity and cellular inheritance. To the chemists at that time and earlier the problem of nucleoprotein was first of all the problem of protein. The structure of the nonprotein portions of nucleoprotein appeared too simple to them. It was the protein portion that counted. Avery's discovery set the stage for the rapidly ensuing elaboration of the structure, function and importance of DNA which ten years later led to the development of the Watson–Crick model for the DNA molecule.

Murray Llewellyn Barr (b. 1908) a Canadian physician together with Bertram in 1949 unexpectedly discovered a small stainable body in the nondividing nuclei of females and its absence in those of males. This body is now called sex-chromatin or Barr body, after its senior discoverer. It can be seen in many tissues of females including the epidermis and the oral mucosa and also in the amniotic fluid surrounding female fetuses. Researchers often wondered whether developmental sex deviants had XX or XY constitution. Before the discovery of the Barr body no knowledge was available on this topic. We now know that the Barr body is a heterochromatic X-chromosome that during interphase is completely or for the most part, positively heteropycnotic and condensed. The discovery a few years later of the sex chromatin and the correct human chromosome number was rapidly followed by the discovery of the first human chromosome abnormalities in the late 1950s. These discoveries were followed by a world wide outburst of research on human chromosomes.

Barbara McClintock (b. 1902) in 1950 discovered the **Activator–Dissociation system** in maize. She is a Distinguished Service member of the Genetics Research Unit of the Carnegie Institution at Cold Spring Harbor, New York. She was mentioned earlier for her skillful and persevering cytogenetic work on maize in her earlier years and for her presentation of cytological proof of crossing over in 1931.

In her studies of the Activator Dissociation system McClintock demonstrated that genic expression is intimately related to chromosomal organization In a variable and mutable strain of maize, two loci were shown to be in control of genic action in the course of development. One of the loci called *Activator (Ac)* seemed to be a master locus in that the second locus called *Dissociation (Ds)* was unable to function in its absence Both loci were believed to be blocks of heterochromatin The presence of both loci in the same nucleus gave rise to an increase in spontaneous chromosome breaks and unstable and mutable genic loci But *Ds* also had a second function in the presence of *Ac* It affected genes lying adjacent to it in that they mutated to the recessive condition McClintock discovered several different such gene controlling systems in maize The full significance of McClintock's findings is still not appreciated Similar systems were later found in *Drosophila* and mouse But most important of all her findings led to the epoch making discoveries in bacteria ten years later that revealed an entirely new class of regulatory genes

John Albert Levan (b 1905) a professor of cytology at the University of Lund, Sweden together with the Indonesian born American Joe Hin Tjio in 1950 showed favorable results with their oxyquinoline squash technique in 40 plant species Together they first worked out the importance of this chemical agent for chromosome analysis The metaphase chromosomes were contracted, the spindle was destroyed and did not interfere with the spreading of the chromosomes at squashing and many cells were arrested in metaphase, which increased the chance of finding good preparations

Later the two scientists applied the squash technique to human tissue In 1956 they published a paper on the chromosome number of man giving 46 as the 2n number Their counts were made from tissue culture preparations of lung tissue from four different human embryos With previously used techniques it had been extremely difficult to make counts in human material Until then the human chromosome number was assumed to be $2n = 48$ Tjio and Levan's demonstration was soon verified by several other research workers As a matter of fact, two English investigators a few months later reported 46 chromosomes in testicular preparations of three adults This represented the basis for cytogenetic research in man, and vertebrates a field of investigation that has developed with an avalanche like rapidity

In the following years the Turner's, Klinefelter's and Down's syndromes were linked to chromosome aneuploidy Levan's special scientific interest is chromosomes in relation to cancer In 1956 he reported 70 to 80 chromosomes in two highly malignant effusions of lung and stomach carcinoma of man Recently (1966) he studied the nonrandom representation of chromosomes in tumor stem cell lines from 40 human cancers and concluded that chromosomes of the C group were over represented while those of D and G groups were under represented.

Sir Francis Harry Compton Crick (b 1916) British biophysicist and geneticist and Kieckhefer Research Professor at the Salk Institute at San Diego, California, in 1953 with the American James Dewey Watson, published a paper in which they proposed a model for the molecular structure of DNA The model they proposed

is now widely known as the Watson-Crick model For the discovery of the molecular structure of DNA and its significance for the transfer of information in living material they received the 1962 Nobel Prize in medicine and physiology together with Maurice Hugh Frederick Wilkins from New Zealand

The discovery of the double helix structures of DNA was based on the achievements of Wilkins and his colleagues at the Kings College in London They had taken good x ray diffraction pictures and had analyzed and interpreted the photographs Watson and Crick had made the brilliant deductions that revealed the structure of the molecule This model of DNA proved immediately fruitful Its structure and the theory of its replication was so clear and uncomplicated that geneticists at once accepted it All investigations since this discovery supported it Enzymatic synthesis of RNA and DNA followed in the 1950s, and by 1961 Crick and his coworkers in an ingenious experiment furnished evidence of the triplet nature of the **codon**, the smallest combination of bases in a polynucleotide, which determines that a specific amino acid shall be inserted at a specific position into a polypeptide chain

The discovery of the triplet genetic code is based on the work of Crick and Nirenberg as an answer to the problem of designating 20 amino acids by a nucleotide code consisting of only four characters In 1966 Crick advanced the **wobble hypothesis**, which was proposed to provide rules for the pairing of a codon in messenger RNA and for an anticodon in transfer RNA of the third position of the codon, degeneracy of the code was explained by this hypothesis The first 2 positions of the triplet codon on messenger RNA pair precisely with the anticodon on transfer RNA but pairing of the third position may be wobbly, and independent of the nucleotide that is present at the third position

Francois Jacob (b 1920), Chief of the Department of Microbial Genetics at the Pasteur Institute in Paris France, together with Jacques Monod in 1961 published a classic paper on the regulation of protein synthesis through which they introduced many new concepts into genetics and established others that had been debated for several years by researchers Together with Andre Lwoff they received the 1965 Nobel Prize in medicine and physiology for their discovery of a previously unknown class of genes whose function is to regulate the activity of other genes

In their concept there is an interplay between three kinds of genes, **structural genes, operator genes,** and **regulator genes.** The structural gene corresponds to the classical gene, possessing the ability to synthesize a specific protein or enzyme that has a special task during the life and development of the individual It would, however, be inconvenient if this enzyme production occurred all the time It would be advantageous if it were stopped and started again when necessary, this activity is controlled by the operator and regulator genes The operator gene apparently is located in immediate proximity to the structural gene and represents something like a switch mechanism that either turns on or shuts off the activity of the structural gene The operator gene, however, does not know when this should be done, but receives orders from a specific regulator gene This gene may not necessarily be located close to the operator gene, but may be located in another part of the chromosome The regulator gene gives its orders via a repressor product that inter-

acts with the operator gene to shut it off under conditions in which the structural gene products are not needed

Mary Francis Lyon, Head of the Genetics Section of the Medical Research Council Radiobiology Unit at Berkshire England in 1961 independently from L B Russel's work during the same year developed the **single active X hypothesis** of dosage compensation in man and mammals known as the *Lyon Hypothesis* Lyon worked with mice She provided evidence suggesting that one X chromosome is inactivated in some early embryonic cells and their descendants that the other is inactivated in the rest and that females are consequently X chromosome mosaics This is a specific manifestation of a much wider biological phenomenon the inactivity of whole chromosome sets specific chromosomes or specific chromosomal regions Lyon's genetic findings verified the cytological discovery by Barr of small stainable bodies in the female nuclei of nondividing nerve cells in cats This stainable body is one of the two X chromosomes that is genetically inactivated by heterochromatinization

Wolfgang Beermann (b 1921) Director of the Max Planck Institute of Biology at Tübingen Germany in 1961 demonstrated that a puffing locus on a polytene chromosome of a dipteran insect *Chironomus* is the site of a gene These puffs arise at different points on these chromosomes and many are found only in specific tissues but vary within a tissue at different times The present view is that the puff signifies RNA synthesis and this view is supported by experiments that stain RNA differentially and show its localization in the puff

Sol Spiegelman (b 1914) professor of microbiology at the University of Illinois in 1961 together with B D Hall demonstrated that hybrid molecules can be formed containing one single stranded DNA and one RNA molecule that are complementary in base sequence This technique opened the way to the isolation and characterization of different kinds of RNA **Nucleic acid hybridization** has since been exploited to study the cell's mechanism for manufacturing proteins One strand of nucleic acid will combine with another wherever the subunits of the two strands are complementary Artificial hybrid combinations clarify the flow of information in the living cell It is now known that the actual synthesis of protein molecules is accomplished with the help of ribosomes which serve as workbenches of protein synthesis in the cytoplasm and evidently hold the translatable RNA in position while the message is being read In 1965 Spiegelman together with Ritossa showed that the genes producing the ribosomal RNA of *Drosophila* are located in the nucleolus organizer regions of the chromosomes It appears now that the precursor material or ribosomal RNA is manufactured by the nucleolus organizer and is then transferred to the nucleolus for final assembly into ribosomes These findings are in line with recent research that indicates that living organisms cannot exist without nucleolar organizer chromosomes

James Bonner (b 1910) a professor of biology at the California Institute of Technology in 1962 together with R C Huang studied the protein components of chromosomes and found that in some cases the rates at which messenger RNA

was produced could be increased by removing histones This pointed strongly to the involvement of histones in regulating gene action If such a mechanism exists, some proteins could serve as locks inhibiting the action of certain nucleic acid molecules Every cell, regardless of its level of specialization, could still contain all genes but each might possess its own kinds of regulating proteins that would block out certain genes in certain cells

Margit M. K. Nass (b 1931) and **Sylvan Nass** (b 1929), a research couple from the Department of Therapeutic Research, University of Pennsylvania, School of Medicine and of the Department of Molecular Biology at the Eastern Pennsylvania Psychiatric Institute, Philadelphia, in 1962 and 1963 furnished one of the earliest reliable reports of mitochondrial DNA
Under the electron microscope they observed fibrous DNase-sensitive regions in thin sectioned chick mitochondria In the same year (1962) Ris and Plaut demonstrated by electron microscope and cytochemical methods that chloroplasts in the plant *Chlamydomonas moewusii* contained DNA Other cytochemical tests had preceded these observations in mitochondria and chloroplasts, but they had not been as conclusive as the ones mentioned The demonstrations under the electron microscope initiated a search in many laboratories that confirmed these findings When viewed with the electron microscope, these so-called **extranuclear chromosomes** differ from nuclear chromosomes of the same cells by their closer resemblance to pure DNA In general, they tend to carry much less protein, are believed to lack histone, and in these and other respects are similar in organization to bacterial or viral chromosomes Even the total amounts of DNA per mitochondrion or per chloroplast are similar to the amount per cell in bacteria such as *Escherichia coli* The study of extrachromosomal or cytoplasmic inheritance has entered a new phase through these new and interesting discoveries

Henry Harris (b 1925), a professor of pathology and Head of the Department of Cell Biology at the University of Oxford, England, together with Watkins in 1965 developed a technique that uses appropriate viruses to cause somatic cells of very different origins, such as from species of different genera, to fuse into one binucleate cell The method is now generally referred to as **somatic cell hybridization** or **cell fusion** Hybridization between somatic cells in vitro promises to provide the basis for cytogenetic analysis of somatic cells in culture The assignment of genes to specific chromosomes is perhaps the simplest and most immediately achievable goal to the analysis of hybrid lines formed by fusing different cells These pioneering studies that are now being conducted by several laboratories around the world have led to rapid advances in the knowledge of the human chromosome map Harris and his colleagues are now heavily engaged in the analysis of malignancy by cell fusion

Ernest Joseph DuPraw (b 1931), a professor of anatomy, who was engaged in clinical training and research at Stanford University School of Medicine, in 1965 and 1966 published techniques of spreading interphase and metaphase chromosomes for electron microscopy and contributed new ideas on chromosome structure His method of **whole mount electron microscopy** involves growing leuco-

cytes or other cells in culture They are blocked at metaphase by using colchicine to disaggregate the mitotic apparatus The blocked cells are spread on an air-water interface that bursts them and releases the chromosomes Finally, the intact chromosomes and nuclei are picked up on electron microscope grids washed or treated with analytical reagents, and dried from liquid CO_2 by the critical point method

DuPraw studied honey bee and human chromosomes by this method Honey bee chromosomes are well suited for this approach because they are extremely small and rod shaped Whole mount electron microscopy has an advantage over thin sectioning because thin sectioned chromosomes are much more difficult to interpret with respect to fiber configurations and dimensions, and there has been wide disagreement among published estimates of fiber diameters DuPraw s interpretation of chromosomal organization is called the **folded fiber model.** Before interphase replication each chromosome is thought to consist of a **unit chromatid,** that is a single long 20 nm to 50 nm fiber that contains a DNA double helix in super coiled configuration DuPraw was only one of many workers who were trying to solve the puzzle of the molecular structure of the chromosome

Tobjörn Oskar Caspersson (b 1910), Swedish cell biologist, who was mentioned earlier for his pioneering studies of nucleic acids, (see p 21) in 1968 along with his colleagues, was the first to demonstrate that when metaphase chromosomes are stained with quinacrine mustard or related substances and examined by fluorescence microscopy, each pair stains in a specific pattern of dark and light bands called **Q-bands.** This revived a whole new search for methods that permit distinction between individual metaphase chromosomes and chromosome segments

One of the more prominent and simpler, new methods developed in the beginning of the 1970s is the **Giemsa staining method.** When chromosomes are treated with a denaturing agent such as trypsin and then stained with Giemsa stain, they take up stain patterns of dark and light bands, called **G-bands,** very similar but not identical to Q bands Giemsa banding is simpler and less expensive than fluorescent banding and provides much the same information, so it will probably become more widely used Chromosome banding techniques have greatly broadened the usefulness of chromosome analysis in cytogenetics Now that metaphase chromosomes can be individually identified, chromosomal rearrangements can be more easily recognized, and the chromosomes involved can be specifically identified As a consequence, mapping of genes on chromosomes is facilitated

Daniel Nathans (b 1928) a professor of microbiology at Johns Hopkins University, Baltimore, Maryland, and his coworkers in 1973 published a paper on the use of **restriction enzymes** for chromosome mapping (Danna et al , 1973) They set the stage for an explosion of research on **restriction maps, transcript maps,** and **nucleotide sequencing** of isolated restriction fragments The so-called **restriction effect** was first discovered by Dussoix and Arber in 1962 They demonstrated that restriction enzymes act like chemical knives that cut DNA strands into defined fragments In 1970 Hamilton O Smith and coworkers reported the purification and characterization of a **specific restriction endonuclease** of the type II (Kelly and Smith, 1970)

Restriction enzymes belong to two different types according to their restriction products Type I cuts at unique DNA sites resulting in specific fragments with unique terminal sequences The cuts are within sequences that show twofold symmetry around a given reference point $\left(\text{e.g., } \frac{\text{AAG CTT}}{\text{TTC GAA}}\right)$ Type II restriction endonucleases are smaller and simpler in subunit composition than type I and are more specific in their cleavage sites Nathans, Smith, and Arber were the winners of the 1978 Nobel Prize for Medicine Arber was credited with having first predicted the existence of restriction enzymes, Smith with having isolated the first such enzyme and Nathans with having first applied these enzymes to the study of genetics Restriction enzymes have become a very important tool in the study of DNA They allow the isolation of sufficiently short DNA fragments and the sequencing of their nucleotides

Stanley Norman Cohen (b 1917), Department of Medicine, Stanford University School of Medicine in Stanford California, in 1973, together with A C Y Chang developed the technique of **DNA cloning** by which DNA molecules from prokaryotic and eukaryotic sources can be spliced together via plasmid vehicles (Cohen et al., 1973) They isolated DNA pieces from the bacterium *Staphalococcus* and spliced them into nonconjugal plasmids Such resulting **recombinant plasmids** in turn were then introduced into *Escherichia coli* Once the isolated DNA segment is incorporated in the *E coli* bacterium, it can be reproduced therein to provide researchers with enough recombinant DNA to determine the exact sequence of the nucleotides

The potential usefulness of such genetic manipulation lies in the fact that in bypassing the sexual cycle a new genetic combination of inherited properties is established Large amounts of a particular gene or combination of several genes can be obtained for study by this method Cloning individual eukaryotic genes with their adjoining control elements could reveal the process of **gene expression** in eukaryotes which has been very difficult to study because of the enormous complexity of the eukaryotic genome For instance a λ phage-mouse β hemoglobin chromosome was constructed and it was discovered that so-called **intervening sequences** in the mouse chromosome about 550 nucleotides in length do not code for β globin at all (Leder et al 1977) Intervening sequences have also been found in rabbit globin genes in genes corresponding to *Drosophila* 28S ribosomal RNA, adenovirus, Simian virus 40 mouse immunoglobin yeast tRNA, and chicken ovalbumin and appear to be a common occurrence of eukaryotic gene organization (Leder et al., 1978) Cloning is considered by some geneticists to be of potential major medical and agricultural benefit Insulin genes have been spliced into bacteria (Villa Komarov et al., 1978), and work that will introduce nitrogen fixing genes *(nif)* into genomes of crop plants is in progress (Streicher and Valentine, 1977)

Charles Allen Thomas Jr. (b 1927) Department of Cellular Biology, Scripps Clinical and Research Foundation La Jolla California, in 1974 together with D A Wilson discovered the widespread occurrence of the so-called **palindromes**, hair pin like structures resulting from inverted **repetitious** DNA which is located at

intervals along the chromatids of eukaryotic chromosomes The name "palindromes" applies because these sequences read the same both backward and forward $\left(\text{e g , } \begin{matrix} \text{ATCTA} \\ \text{TAGAT} \end{matrix} \text{ t*} \right)$ Palindromes may be miniature "handles" that could be useful in the dissection of chromosomes Boyer (1974) reported that many sites recognized by restriction endonucleases prove to be palindromes Many palindromes, particularly those recognized by restriction enzymes, are only 3 to 10 base pairs long Longer ones are hundreds of base pairs in size

This short history has shown some of the trends in cytology, genetics, and cytogenetics during the last four hundred years It demonstrates the close interdependence of tool development, the imagination of people, and the art of integrating bits of information into a framework of facts and working hypotheses From the early microscope builders, who saw the first cells and discovered some of the first principles of life, to the sophisticated researchers of the 20th century, who have the most advanced technology at their disposal, it is a story of fascinating development that can be read from the lives and ambitions of many devoted scientists

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*t stands for turn-around region, the point where the single linear DNA chain folds back

Part II
Structure of Chromosomes

Chapter 2
Gross Morphology of Chromosomes

This chapter emphasizes the aspects of gross morphology of chromosomes that are visible under the light microscope. In Chapter 3 aspects of fine structure will be discussed

There are several stages at which chromosomes can be studied, and each stage has advantages and disadvantages. The stage of the cell cycle in which the chromosomes are most easily identified and distinguished is during **mitotic metaphase** when they are usually most condensed or coiled. In the past, methods for preparing mitotic metaphase chromosomes did not reveal many morphological characteristics that could be used to distinguish them within the complement. Only a few criteria could be employed to describe them. Due to the lack of simple and reproducible differential staining procedures for such ordinary metaphase chromosomes, cytologists turned their major attention to special chromosome types such as the **giant salivary gland chromosomes** of insects and some other organisms that exist in the prophase stage. Because of their polyteny—an increase in lateral multiplicity—they reveal much detail that usually cannot be studied in ordinary prophases. Other advantages of the study of prophase are (1) the possibility to distinguish between eu- and heterochromatin, (2) the visibility of chromomeres, and (3) the presence of nucleoli that are associated with specific chromosomes and that mark them as **nucleolus organizer chromosomes**. For these reasons many species have been subject to **pachytene analysis**. But there are disadvantages to the morphological study of the pachytene chromosomes of meiosis. Because of their considerable length, they are not usually all visible in squash preparations. The higher the **n**-number of chromosomes, the more difficult is a pachytene analysis.

However not every organism can be analyzed in this manner. Those scientists who worked on the majority of species, including man, had to rely on the ordinary metaphase chromosome analysis. But a recent major breakthrough in cytogenetic technology has suddenly changed this situation (see Chapter 1). Several reliable methods are now available that reveal unique **banding patterns** in mitotic metaphase chromosomes.

In 1971 an ad hoc committee meeting on the Standardization of Human Chromosomes was held to revise the nomenclature system in light of new techniques and new findings (Paris Conference, 1971). This system of cytogenetic human nomenclature was again revised in 1978 (International System, 1978). The Paris Conference describes four different chromosome banding methods now known as

C-banding, G banding Q banding, and R-banding In this chapter we will consider the different applications for studying the gross morphology of chromosomes

2.1 Mitotic Metaphase Chromosomes

Because of the recentness of the discovery of banding patterns in mitotic metaphase chromosomes the majority of metaphase chromosome analyses have been carried out with the aid of other methods It is therefore important for the student of chromosome morphology to familiarize himself with earlier approaches Mitotic meta phase chromosomes usually range in sizes from about 0 5 μm to 30 μm in length and from 0 2 μm to 3 μm in diameter Plants and animals alike can have very small chromosomes but on the average plants have larger chromosomes than animals

2 1 1 Total Length of Chromosomes

The morphology of a chromosome in mitotic metaphase is described by two major factors its total length and the position of the centromere In order to demonstrate these characteristics, cytologists construct **idiograms** of the **karyotypes** of species The karyotype as described by Battaglia (1952) is the particular chromosome com plement of an individual or a related group of individuals, as defined by chromosome size, morphology, and number An idiogram is a diagrammatic representation of the gametic chromosome set (n) of a given species and is used to compare the karyotype of one species with those of other species Figure 2 1 shows an idiogram of *Agropyron orientale* (Schulz-Schaeffer and Jurasits, 1962) There exist karyotypes with chromosomes essentially similar in size and others with chromosomes differing greatly in size The average size of chromosomes is 6 μm The longest chromosomes exist in the plant genus *Trillium* and are longer than 30 μm The shortest chromosomes are less than 1 μm in length and occur in fungi, rushes, sedges, and in some animals In many species we find two distinct sizes of chromosomes, large ones and small ones Such karyotypes occur in the plant genera *Yucca* and *Haemanthus* (Fig 2 2) and in birds and lizards In polyploid plant species, groups of chromosomes in different size classes give clues of parental origin For instance, in the grass genus *Bromus* the North American octoploids

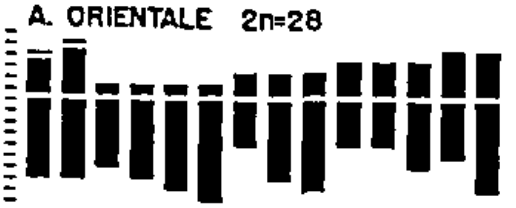


Fig 2 1 Idiogram of *Agropyron orientale* (2n = 28) The satellite chromosomes are placed at the beginning of the idiogram and are arranged according to the length of their satellites The rest of the chromosomes are arranged according to the length of their short arms One unit of the scale to the left of the idiogram equals 0 72 μm (From Schulz-Schaeffer and Jurasits, 1962 Reprinted by permission of McClure Newspapers Inc, Burlington Vermont)

Fig 2 2 Mitotic metaphase chromosomes of the plant *Haemanthus katharinae* (2n = 18) × 2000 (Courtesy of Dr A H Sparrow, Biology Department, Brookhaven National Laboratory, Upton, New York)

(AABBCCLL) have 6 **basic genomes*** (6x) of medium size chromosomes and 2 basic genomes (2x) of long chromosomes According to genome analysis by Stebbins (Stebbins and Tobgy, 1944, Stebbins 1947a), the medium size chromosomes are homologous with the chromosomes of the hexaploid species of section Ceratochloa confined to South America (AABBCC) while the long chromosomes (LL) are homologous with those of the North American diploids of section Bromopsis Similar homologies exist between the genomes of Old World and New World cottons (Skovsted, 1934)

2 1 2 The Centromere

Centromeres could be classified as follows

1 Localized centromeres
2 Neocentromeres
3 Nonlocalized centromeres
  a Polycentromeres
  b Holocentromeres

The **localized centromere** constitutes the normal condition in which a chromosome possesses a permanently localized region to which the spindle fiber attaches during chromosome movement **Neocentromeres** form under certain conditions in which the centromere region is replaced by a secondary center of movement These are exceptional cases in which the chromosome ends move first during anaphase of

*Basic genome A group of chromosomes that are thought to have been present in the gametes of the diploid ancestors of polyploids and those groups that are present in the gametes of the still existing diploids of a genus The number of chromosomes in a basic genome is represented by the **basic chromosome number** or **x—number** (x = 7 in *Bromus*)

meiosis (Rhoades 1952) **Nonlocalized centromeres** are those in which the spindle attachment is not confined to a strictly localized chromosome area In the case of **polycentromeres,** each chromosome is attached by many spindle fibers Here many centromeres are separated by noncentric segments Examples of this type of centromere are some ascarid nematodes **Holocentromeres** (Hughes-Schrader and Ris 1941) are diffuse in nature where every point along the chromosome shows centromeric activity Such centromeres have been observed in Hemiptera Homoptera Protista and the higher plant genus *Luzula*

The site of the localized centromere is often referred to as the primary constriction or kinetochore Its location on the chromosome is probably the most important character in determining the morphology of the chromosome The centromere is observed as a constriction in the metaphase chromosome and is stained lighter than other parts of the chromosome This constriction can be located toward the end of the chromosome in the center or in between According to its position it will subdivide the chromosome into 2 equally or unequally sized arms Chromosomes are categorized according to the position of the centromere as **telocentric, subtelocentric, submetacentric,** and **metacentric** chromosomes

Chromosome arms formed by the location of the centromere can be measured and their lengths expressed in different ways A very popular nomenclature for expressing these measurements is the one used by the Human Chromosome Study Group (Chicago Conference 1966) This nomenclature designates the short arm with the letter "p" (abbreviation for *petit* French for *short*) and the long arm with the letter 'q ' The ratio between the arms is often calculated as the arm ratio

$$A = \frac{p}{q}$$

or as the centromeric index (Chicago Conference 1966)

$$C = \frac{p \times 100}{p + q}$$

Other indices or formulas have been used also

Telocentric chromosomes are those with a terminally located centromere Telocentric chromosomes may arise by centromere misdivision or breakage induced within the centromere region Telocentrics are generally considered to be unstable since fracturing of the centromere is usually involved The instability of telocentrics is considered to be the reason for their rarity in nature

Most chromosomes are **monocentric,** having only one centromere per chromosome Chromosomes with two centromeres are called **dicentric** Such dicentric chromosomes are usually the product of structural changes Dicentric chromosomes may pass through cell divisions without difficulty But it may happen that the two centromeres pass to opposite poles which causes bridge formation If such bridges break each daughter nucleus will contain two broken chromatid ends Freshly bro-

ken chromatid ends have the tendency to fuse, which in this case will result in newly formed dicentrics A similar cycle may happen during the next division McClintock (1938a, 1938b, 1941a, 1941b, 1941c, 1942, 1944) studied this chromosome behavior in maize and called it the **breakage-fusion-bridge cycle** (see Chapter 11) The irregular behavior of dicentric chromosomes must be the reason that they normally do not occur in nature and cannot generally be maintained in laboratory or field stocks of most organisms However, a transmissible dicentric chromosome was discovered by Sears and Camara (1952) in wheat, *Triticum aestivum* L The reason for the transmissibility must be the partial inactivity of the second centromere As long as one of the two centromeres has stronger centromeric activity than the other, it can cause the chromosome to be pulled to one pole in its entirety without tearing Other forms of centromeric activity in chromosomes are of the neocentric and diffuse kind But since they are not localized, they do not contribute to the morphology of the chromosome They therefore will be discussed later

2 1 3 The Nucleolus Organizer Region

In addition to a primary constriction formed by the centromere, certain chromosomes reveal a region that is called **secondary constriction.** This region is responsible for the formation of the nucleolus during telophase and is associated with this structure during interphase and prophase, it is therefore called the **nucleolus organizer region** (McClintock 1934) It is also the chromosomal site of ribosomal RNA synthesis as mentioned in Chapter 1 (see Spiegelman, p 25) However, in metaphase the nucleolus is generally not visible This region in its true sense is really not a constriction like the centromere since its diameter is mostly as great as the remainder of the chromosome But the region is usually very strikingly marked since it is negatively heteropycnotic to such a degree that the remaining portion of the chromosome, the so-called **satellite,** seems to be removed from the rest of the chromosome like a chromosome fragment Each species usually possesses at least one homologous pair of **nucleolus organizer chromosomes,** a pair that has a nucleolus organizer region Very often, each basic genome (x) has such a pair of nucleolus organizer or **satellite chromosomes.** In the genus *Bromus* 31 species were investigated that represented 166 basic genomes The number of satellite chromosomes found were 160, almost coinciding with the number of basic genomes (Schulz Schaeffer, 1960) In this way, satellite chromosomes can serve as marker chromosomes for specific basic genomes, and they are, therefore, valuable for cytotaxonomic studies The size of the satellite can vary considerably Satellites are generally attached to the short arm of a nucleolus organizer chromosome In the human chromosome complement, the so-called **acrocentric** (subtelocentric) chromosomes of the D and G groups all have tiny satellites that are so small they are often not manifested in every cell (Fig 2 3) Larger satellites can possess a separate constriction and are then called **tandem satellites** (Taylor, 1926) Satellite type XIV (Fig 2 4) of *Bromus sitchensis* Trin , *B haenkeanus* (Pressl) Kunth, *B coloratus* Steud , and *B valdivianus* R A Phil , of the section

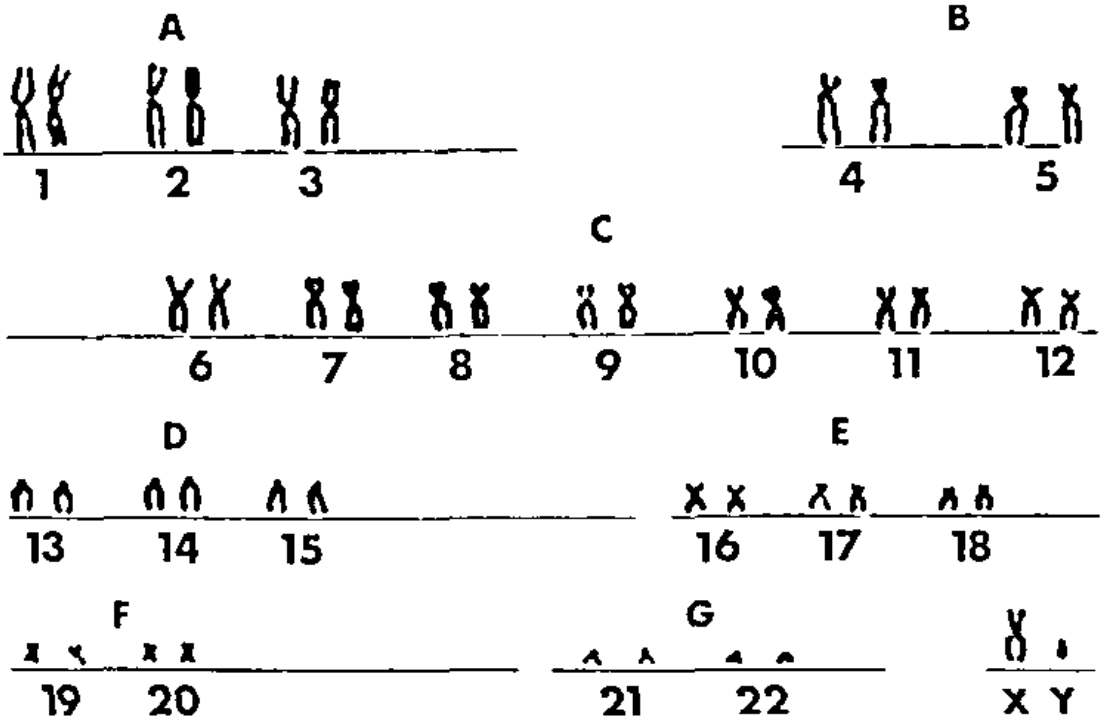


Fig 2 3 Karyotype of human male (Courtesy of Dr Philipp Pallister Shodair Crippled Children Hospital Helena Montana)

Ceratochloa of *Bromus* has such a tandem satellite (Schulz Schaeffer 1960) Sat ellites also may show considerable variation in size This may be correlated to the fact that satellites are mostly believed to be **heterochromatic** Heterochromatin is generally considered to be void of genetic activity found in **euchromatin** Con sequently it is relatively dispensible to the genome However Phillips et al (1977) demonstrated that the gene for *polymitotic (po)* in maize is located in the satellite of chromosome 6 Giant satellites in man were first reported by Tjio et al in 1960 Other so-called secondary constrictions have been detected that are not connected with nucleolus organization The author at one time proposed to call these **tertiary**

Fig 2 4 Satellite chromosomes of the genus *Bromus* arranged according to the length of the satellites One scale unit equals 0 5 μm (From Schulz Schaeffer 1960 Reprinted by permission of the American Genetic Association Washington D C)

2 2.2 The Chromomeres

The **chromomeres** are bead-like projections, along the entire length of a pachytene chromosome, that are heavier stained than the **interchromomeric regions** (Fig 2 5B) They are typical for mitotic and meiotic prophase alike The now almost generally accepted interpretation of chromomeres is that they are structures resulting from local coiling of a continuous DNA thread They probably represent units of DNA replication, RNA synthesis, and RNA processing (Rieger et al , 1976) The heterochromatic chromomeres stain darker than the euchromatic chromomeres They also seem to be larger than the euchromatic chromomeres and have, therefore, been referred to as **macrochromomeres** (Gottschalk, 1954) as opposed to **microchromomeres.** Chromomeres also vary in size within these artificial size classes For instance, the chromomeres next to the centomere are large and become progressively smaller toward the chromosome ends Lima-de-Faria (1952) detected a **chromomere size gradient** that described this progression in diminishing chromomere size He concluded that a detailed pachytene chromosome analysis includes a study of the number, size and disposition of the chromomeres of each chromosome, thus permitting construction of a map of each chromosome type (Fig 2 5A) The number of chromomeres within a pachytene chromosome seems to be reasonably constant and can serve as a reliable morphological characteristic Different methods of constructing pachytene chromosome maps have been applied One method (Gottschalk, 1954) uses a schematic illustration of the chromosome in which the heterochromatin is depicted as a dark bar and the euchromatic portions as a thin line The numbers of the chromomeres in

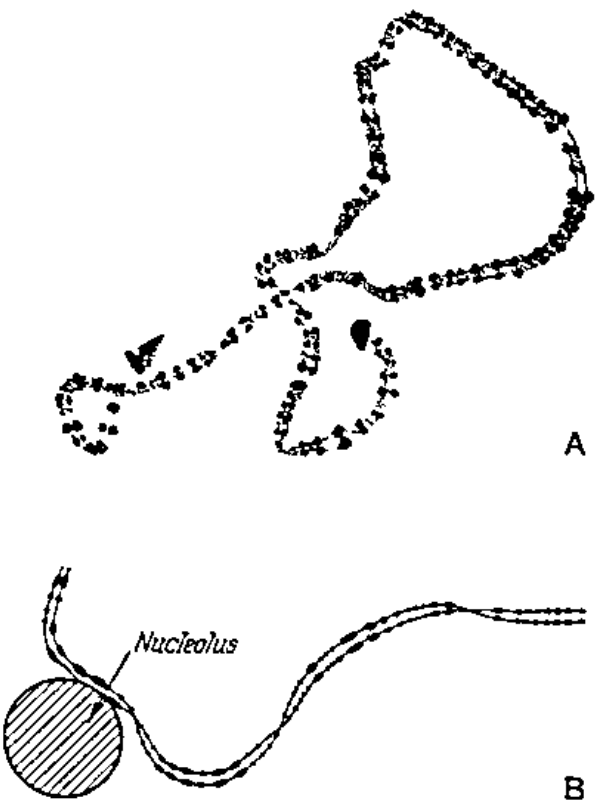


Fig 2 5 (*A*) Pachytene bivalent depicting chromomere size gradient indicating that chromomeres next to centromeres are large and become progressively smaller toward chromosome ends (From Lima de-Faria, 1949 Redrawn by permission of the Mendelian Society, Lund Sweden) (*B*) Pachytene bivalent showing bead like chromosomes (from Rieger et al , 1968)

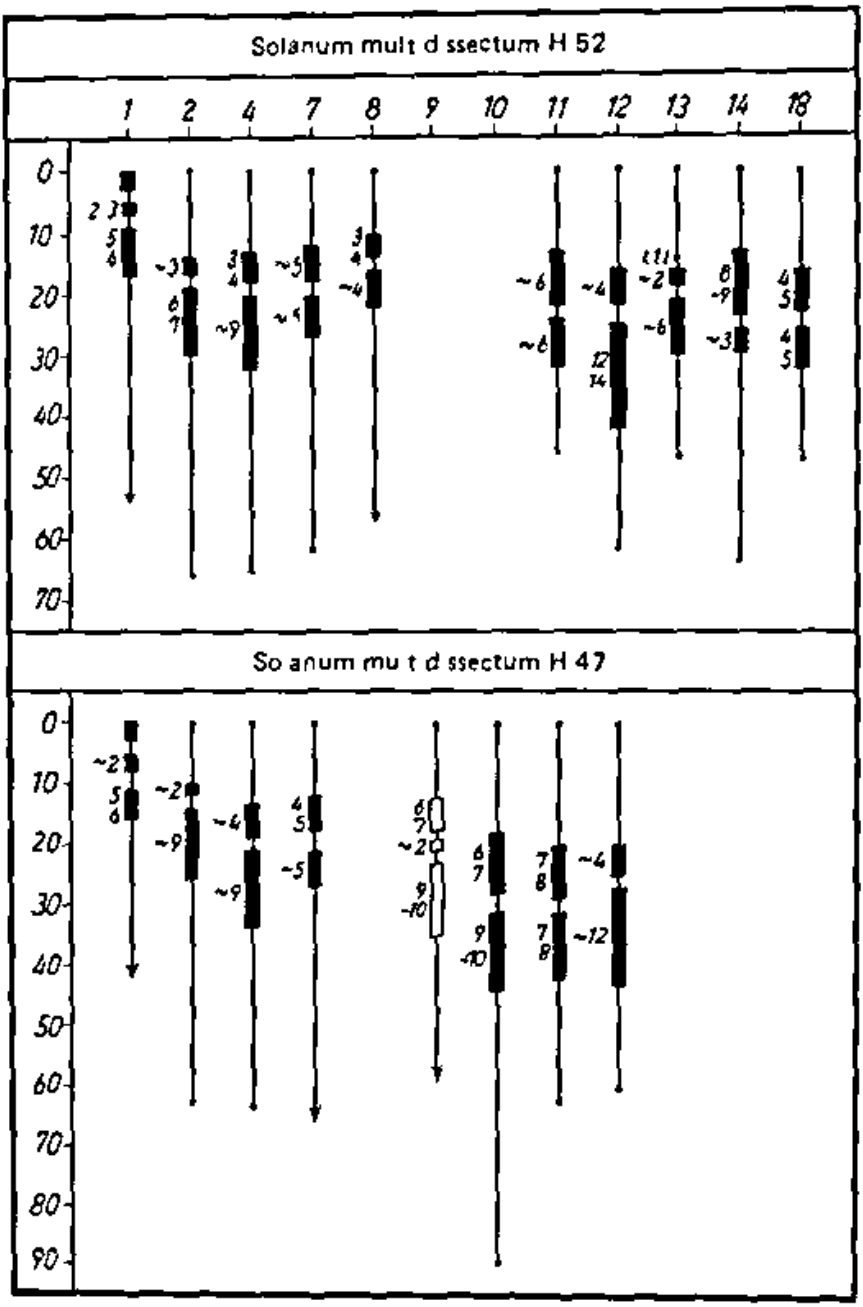


Fig 2 6 Schematic representation of pachytene chromosomes of 2 cultivars of *Solanum multidissectum* The numbers are macrochromomeres in heterochromatin (dark bars) (From Gottschalk and Peters 1956 Redrawn by permission of Verlag Paul Parey Hamburg Germany)

the heterochromatin (macrochromomeres) are given next to the dark bars (Fig 2 6)

2 2 3 The Centromere

Some essential characteristics of the centromere have been already given in Section 2 1 2 Centromeres in pachytene often show up characteristically different from those in mitotic metaphase In many animal and plant species the pachytene cen

tromere consists of one to three chromomere pairs of different sizes, that are connected to the chromosome arms by thin fibers Here again, Lima-de-Faria (1949, 1954) made a careful study of this structure He suggested that the centromere is a compound structure that could be fractured with each broken part still functioning as a separate centromere

2 2 4 The Telomeres

Telomeres are the enlarged terminal chromomeres of chromosomes They seem to be an integral part of chromosomes, just like the centromere in that the chromosomes do not function normally when the telomeres are missing They seem to seal off the ends of normal chromosomes so that they cannot join with other broken chromosome ends In special instances telomeres can have centromeric activity and are then called neocentromeres (Rhoades and Kerr 1949) Lima de-Faria and Sarvella (1958) studied the compound structure of the telomere in several plant species and stated it consists of two separately distinctive regions the **protelomere** and the **eutelomere.** According to their observation the protelomere is a terminal deep staining structure with sharp limits, usually consisting of one to three dark staining large chromomeres The eutelomere is a weakly staining subterminal segment adjacent to the protelomere One compound telomere may consist of as many as eight different chromomeres Parts of such a structure may break off without loss of genetic function of such a structure According to electron-microscopic investigations, the telomere consists of irregularly folded chromatin fibers that rarely terminate at the chromosome ends, but loop back into the chromatid (Rieger et al , 1976)

2.2.5 The Nucleolus Organizer Region

The identification of the **nucleolus organizer region** (NOR) is simplified in the pachytene stage since the nucleolus is in immediate contact with this region at this time of meiotic division Specific chromomeres are recognizable in this region during prophase, and they are called **nucleolus organizer bodies.** In maize, McClintock (1934) demonstrated a heteropycnotic knob in this region on chromosome 6 and showed that this knob is the organizer of the nucleolus As in the case of the centromere and the telomere, this knob is a compound structure After breakage, both fractured portions are capable of forming nucleoli For some reason the smaller portion is able to form the larger of the two nucleoli When only one of the two broken parts of the nucleolus organizer body are present in a cell, it is capable of collecting the entire mass of the nucleolar material More recent studies show the role of this organizer in nucleolus formation (Givens, 1974, Givens and Phillips, 1976) Brown and Gurdon (1964) first demonstrated in the toad, *Xenopus laevis* that the nucleolus is not only the site where ribosomal RNA is accumulated and stored but also the site where it is synthesized They detected a deletion in the nucleolus organizer region of chromosome 12, called *O-nu* Normal *Xenopus* cells (+/+) contained 2 nucleoli but heterozygous cells (+/*O-nu*) only one. Heterozygous toads were viable When two heterozygous toads were hybridized, approx-

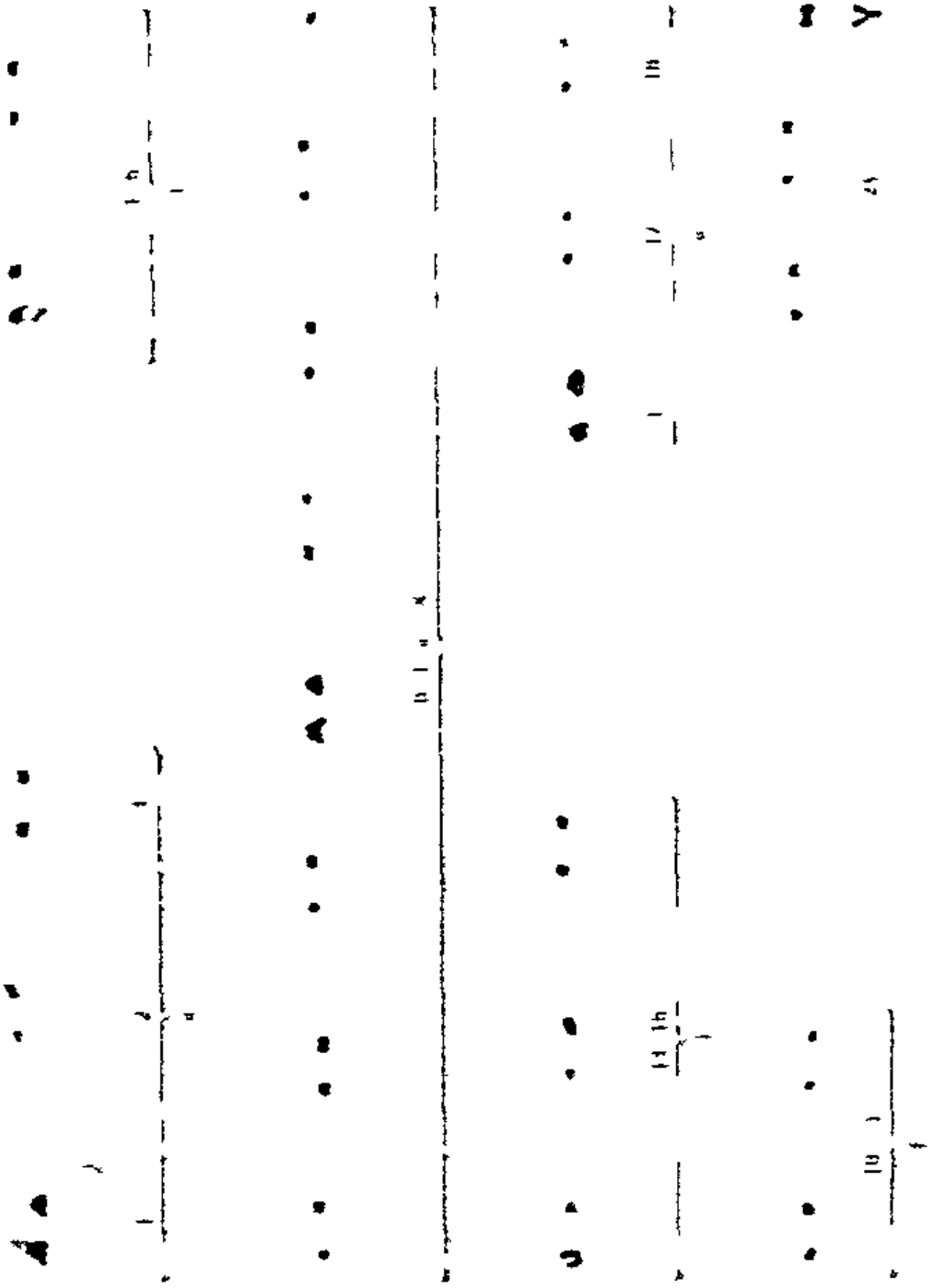


Fig. 2. Karyotype of normal human male after C-banding. (Courtesy of Dr. Cheryl Walker, Louisiana State University Shreveport)

terminated by darker staining during metaphase and prophase. In metaphase it usually does not show up. Therefore, it takes special methods to make heterochromatin visible in metaphase chromosomes. These methods usually involve treatment with acid, alkali, or elevated temperature. It is presumed that cellular DNA is denatured by these treatments. An overnight incubation at 60°C in saline-citrate solution presumably renatures the DNA. Therefore, it seems reasonable that highly repetitious DNA such as constitutive heterochromatin rena-

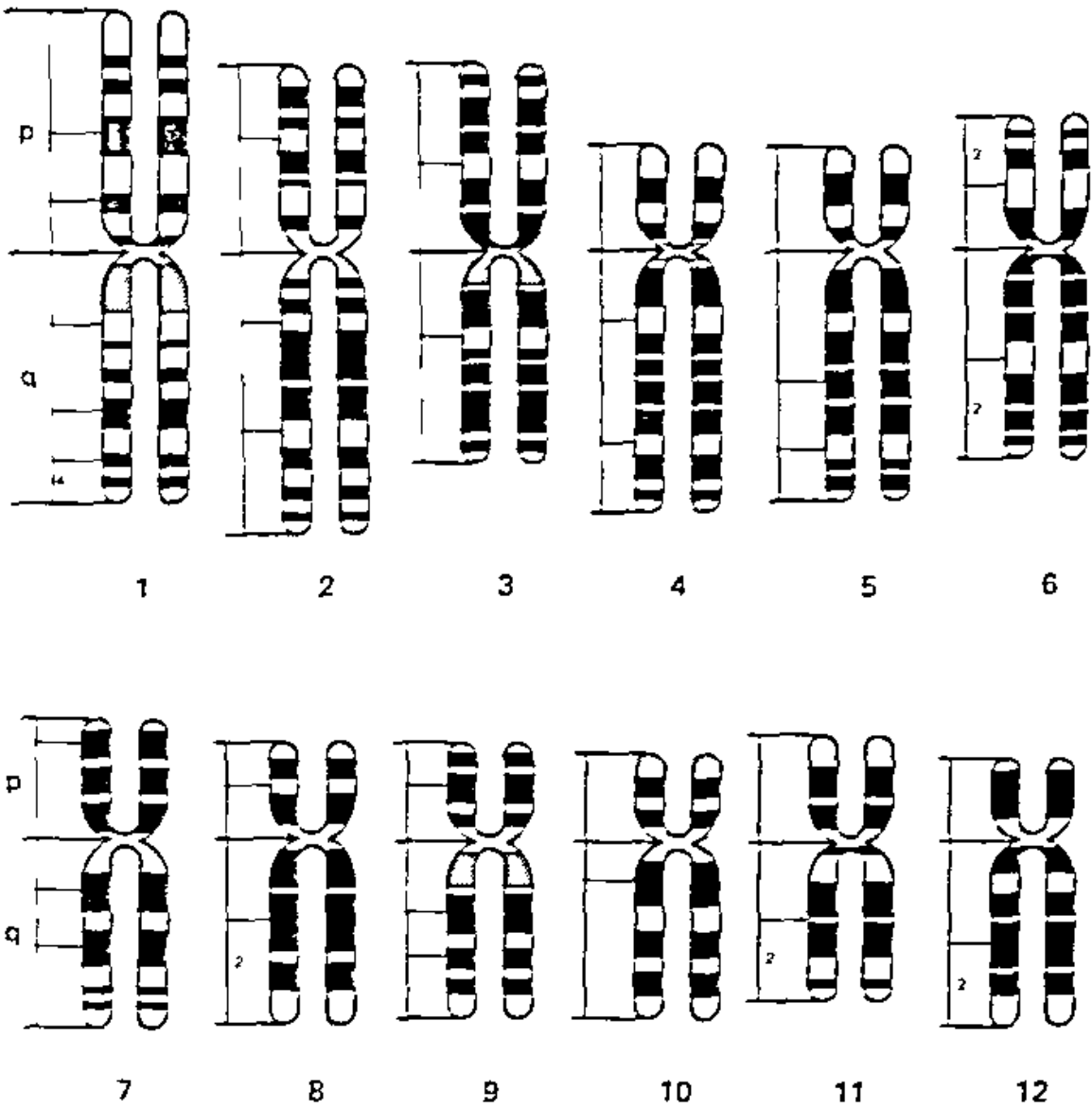
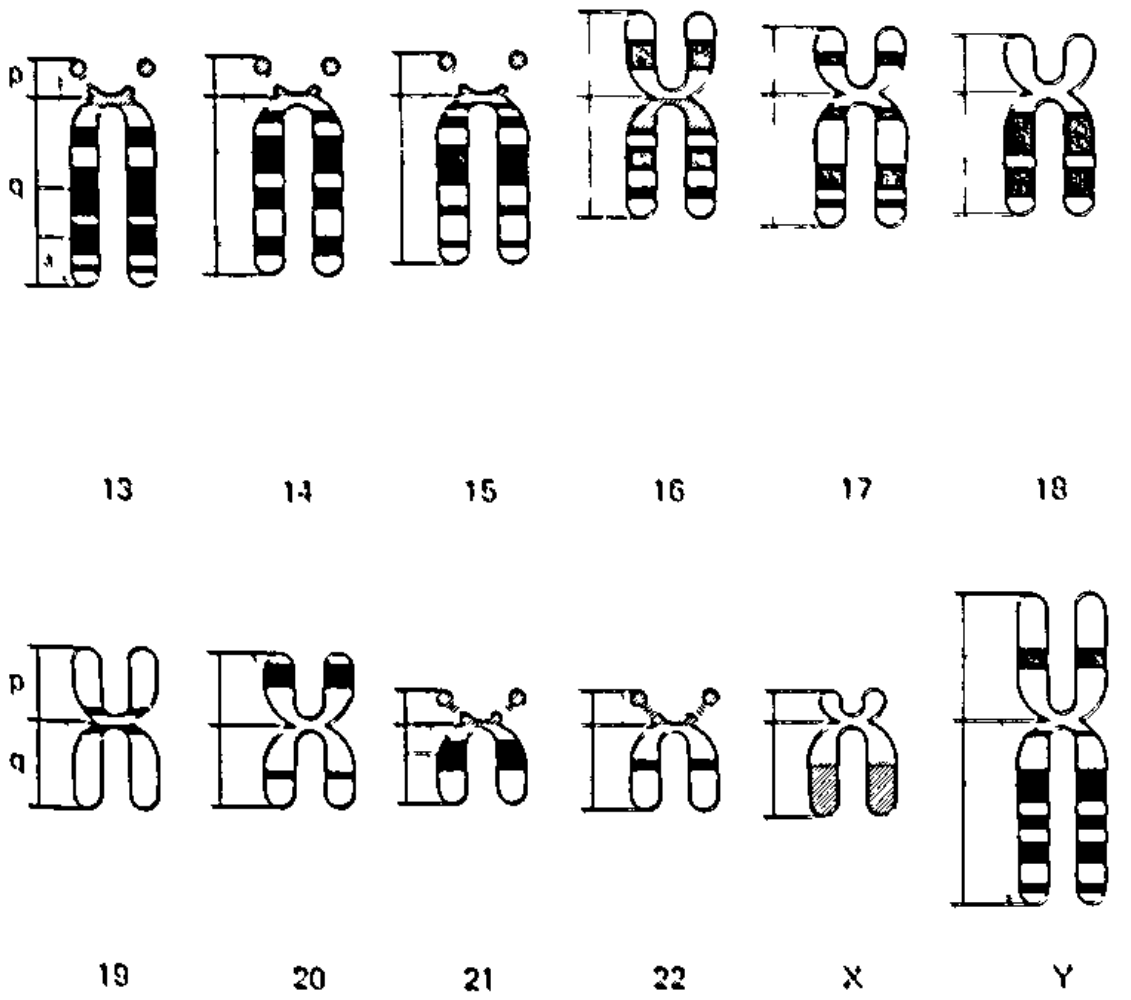


Fig 2 11 Diagrammatic representation of human chromosome bands as observed with the Q- G and P banding methods (From Paris Conference 1971 Reprinted by permission of S Karger A G., New York)

tures under prescribed conditions while low repetitious DNA and unique DNA do not, thereby resulting in the differential staining reaction (Hsu, 1973) In most of the mammalian species constitutive heterochromatin is located in the proximity of the centromere The amount of it in each chromosome seems to be characteristic but in man, polymorphism does occur (Craig Holmes and Shaw 1971 Craig Holmes et al., 1973) Nevertheless, the Human Chromosome Study Group has started to use C banding for the characterization of chromosomes Banding patterns obtained with this method do not permit individual identification of each human chromosome but are helpful in the process (Fig 2 10) The C band technique is very useful for identifying the Y chromosome of mammals which is often entirely heterochromatic particularly in species where its length extends 2 μm (Hsu, 1973) C banding has been also applied to plant material Vosa and Marchi



(1972) demonstrated that plant heterochromatin may show up even more dramatically than animal heterochromatin. For instance Linde-Laursen (1975) tried to explore the extent of **band heteromorphy** in barley by Giemsa C banding in order to evaluate the use of the bands as markers in cytogenetic investigations.

2.3.2 G-Bands

The G banding technique provides more detail than C banding. Along with Q- and R-banding it is ideal for the cytogeneticist since almost every chromosome within

Fig 2.12 Male human G band karyotype (Courtesy of Dr Cheng W Yu, B rth Defect Center Department of Pediatrics, Lousiana State University Med cal Center Shreveport)

demonstrate the nucleolus organizer Along with C-banding, it has proven to be a *superior method for plant material* (*Gerlach* 1977, *Jewell*, 1979)
The popular G-banding method has refused to reveal G-banding patterns in plant chromosomes The absence of G-bands in plants was explained by Greilhuber (1977) He states that plant chromosomes contain much more DNA in metaphase than vertebrate chromosomes of the same length For simple optical reasons vertebrate chromosomes would not show G bands either at such a high degree of contraction

Chapter 3
Fine Structure of Chromosomes

In 1976 Watson wrote that "even today" our fundamental knowledge of the molecular structure of chromosomes is very incomplete This is particularly relevant for the more complex chromosomes of higher plants and animals The main chromosome component of bacteria and viruses is **deoxyribonucleic acid** (DNA) However, up to 50% of the chromosomes of higher organisms is **protein** Information on the ultrastructure of chromosomes has been obtained by various techniques including x ray diffraction, chemical analysis, electron microscopy, and autoradiography

3 1 The Structure of DNA

Deoxyribonucleic acid the genetic material of all cells, is a polymer of deoxyribonucleotides Its primary building block is called the nucleotide and consists of 3 types of simple molecules a phosphate a pentose sugar deoxyribose, and one of four nitrogenous bases The sugar molecules are linked together by the phosphates, and each sugar molecule is attached to a single base The bases are either purine (adenine and guanine) or pyrimidine (cytosine and thymine) bases Nucleotides linked together by phosphatediester bonds form a polynucleotide The secondary structure of DNA has been successfully described by several authors Wilkins and Randall in 1953 concluded from x-ray diffraction studies in sperm heads of the cuttlefish, *Sepia* that the polynucleotide chains of DNA are helical and not extended Watson and Crick (1953a, 1953b), Franklin and Gosling (1953), and Wilkins et al (1953) all came to the conclusion that two helices are present in the DNA molecule As was mentioned in Chapter 1, Watson and Crick (1953a, 1953b) made the brilliant deductions that showed how the two helices fit together They are linked together by hydrogen bonding of the base pairs (thymine-adenine, cytosine guanine) so that each base pair forms a link between the sugar molecule on one helix and the opposite sugar molecule at the same level on the other helix (Watson Crick model) (Fig 3 1) The two right-handed helices are coiled in an interlocked form (plectonemically) about the same axis Each turn or pitch of the so-called double helix includes 10 base pairs (Fig 3 2) When DNA in crystalline form is studied by x ray diffraction, the double helix makes one full turn every 3 4 nm

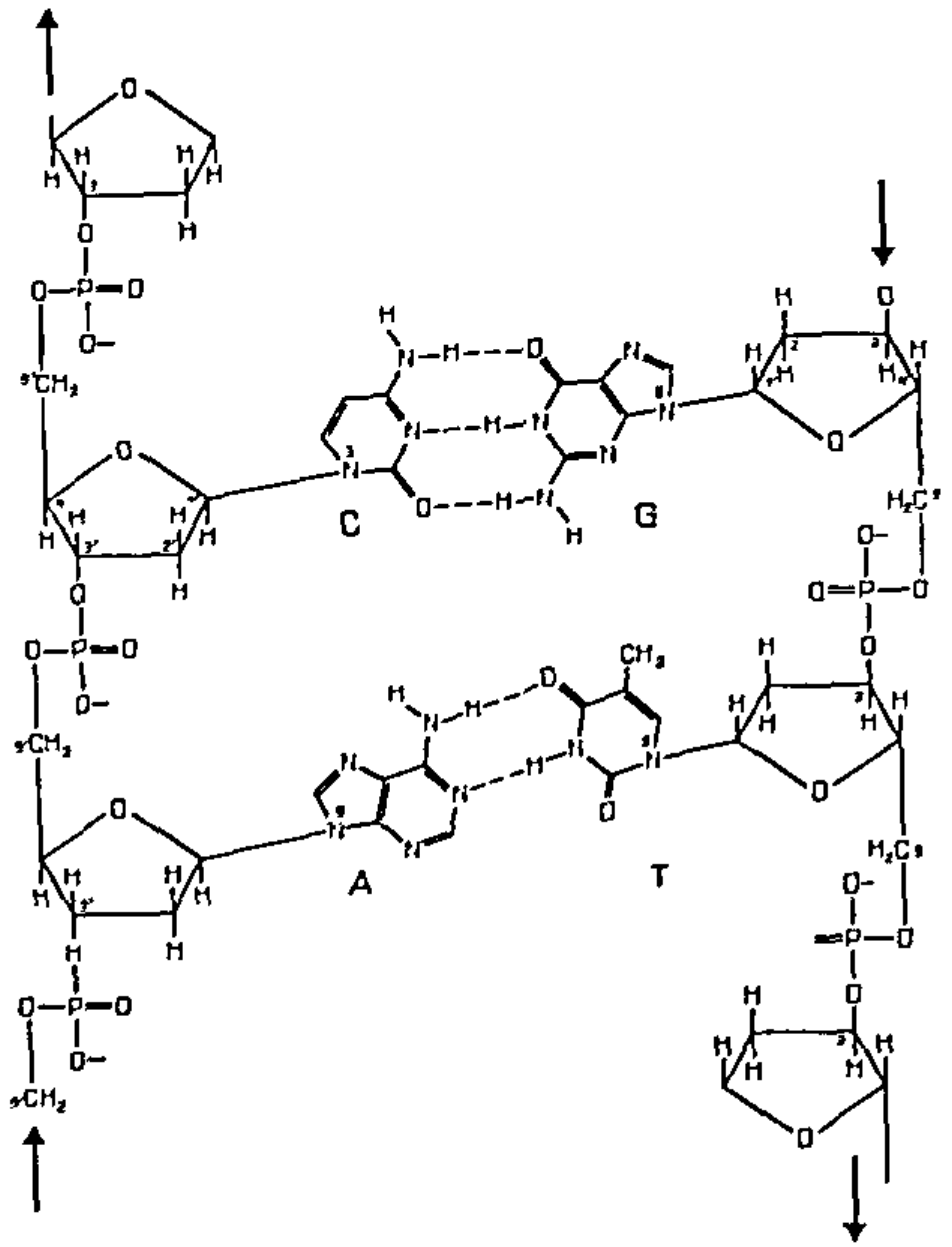


Fig 3 1 A two-dimensional representation of a DNA double helix showing the opposite polarities of the sugar phosphate linkages in the two strands (From Herskowitz, 1967)

3.2 The Structure of RNA

Closely related in structure and function to DNA is ribonucleic acid or RNA DNA and RNA differ in the composition of their pentose The RNA pentose sugar is a ribose instead of a deoxyribose Further, RNA contains no thymine but rather the closely related pyrimidine uracil

In contrast to DNA, the RNA molecules are usually single stranded In connection with the chromosome structure, RNA is important since it is the primary carrier of genetic information in some viruses In these viruses DNA is replaced by RNA The major function of RNA in the cell is to serve as a template substance The

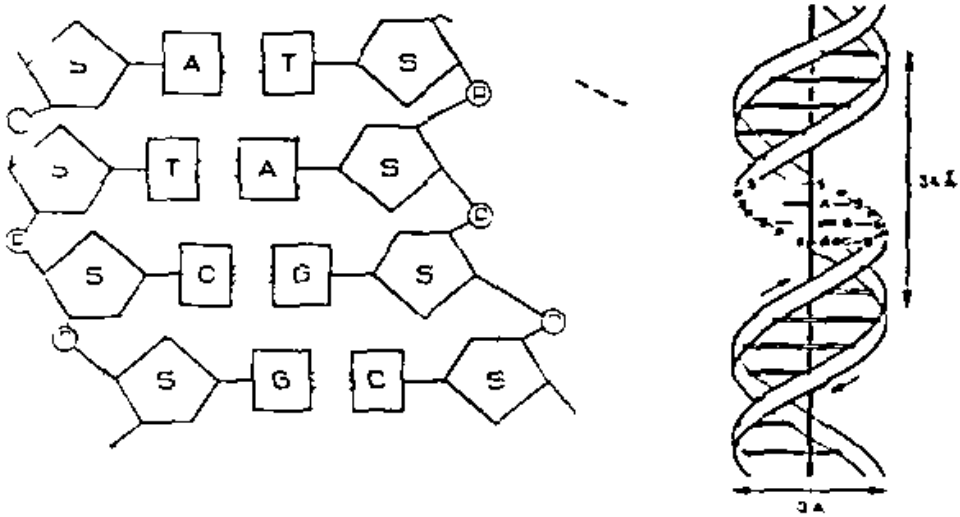


Fig 3 2 Double stranded DNA hel x with the dimens ons of the hel ces ind cated (From Herskowitz, 1967)

template RNAs are mostly called messenger RNA or mRNA Other RNAs in the cell are ribosomal or rRNA and transfer or tRNA

3 3 Nucleoproteins

As mentioned up to 50% of the chromosomes of higher organisms is protein Proteins associated with DNA in the nucleus are basic proteins such as protamine and histone They are of low molecular weight the protamines between 1000 and 5000 the histones between 10 000 and 20 000 Protamines are a component of animal sperm chromosomes Several theories have been advanced about the special relationships of DNA and proteins in the chromosome One earlier theory postulated that a histone α helix fits into the grooves of the DNA double helix (Zubay and Doty 1959) More recently it is believed that the eukaryotic DNA is tightly complexed to proteins and comprises the nucleoprotein fibers called chromatin (Watson 1976) According to electron micrographs this chromatin has a beaded structure and the components of this structure are spheroid chromatin units called **ν bodies** or **nucleosomes** 6 0–8 0 nm in diameter (Olins and Olins 1974 Oudet et al 1975) Olins and Olins and Oudet et al used this name because of the new discovery of these bodies In spite of their different magnitude such nucleosomes are very reminiscent of the chromomeres visible under the light microscope in leptotene and pachytene which have been known since at least 1896 when Wilson described them in the first edition of his book *The Cell in Development and Heredity* (see Chapter 1) However nucleosomes and chromomeres should not be mistaken for one another Each of the nucleosomes or repeating units is believed to have 140 DNA base pairs and eight histone molecules made up of the four main types of histone H2a H2b H3 and H4 (Kornberg 1974) The structure of the ν-bodies has not yet been entirely revealed but the eight histone molecules are believed to fill the central part of the nucleosome The

histones in this structure are believed to be involved in the process of chromosomal contraction Chromosome condensation may be a function of cyclic chemical changes of these histones as they are being phosphorylated, methylated, and acetylated (Watson, 1976)

Vengerov et al (1978) proposed a model for a **nucleosome package** that is a 20 nm globule formed by six nucleosomes (4 to 8 estimated by Müller et al , 1978) of a **nucleosomal fiber** with internucleosomal DNA segments being wound around the nucleosomes in which part of the DNA is covered by histone H1 The diameter of the internucleosomal DNA linkers is 2 nm (Fig 3 3) Such DNA linkers vary in length from about 30 to 70 base pairs (Elgin and Weintraub, 1975) Shelton et al (1978) estimated the size of the circular Simian virus 40 chromosome as being 5,224 base pairs, with a nucleosome size of 187 base pairs, the number of nucleosomes being 22 and the DNA linkers varying in size from 0 to 172 with 23% of them 20 base pairs in size Nucleosomes are actually too small in size to be in the magnitude of actual genes The present idea of gene size is in the order of approximately 1000 DNA base pairs (Goodenough, 1978)

3.4 Models of Chromosome Ultrastructure

Models of the ultrastructure of chromosomes reflect theories based on available data and serve as the basis for further experimentation Data that serve for such models are collected from a wide range of approaches The broad scope of attacking this problem is reflected in a recent symposium on *Chromosome Structure and Function* (Cold Spring Harbor Symposia on Quantitative Biology, 1974) Almost a hundred different ways of *dissecting* the chromosome are represented,

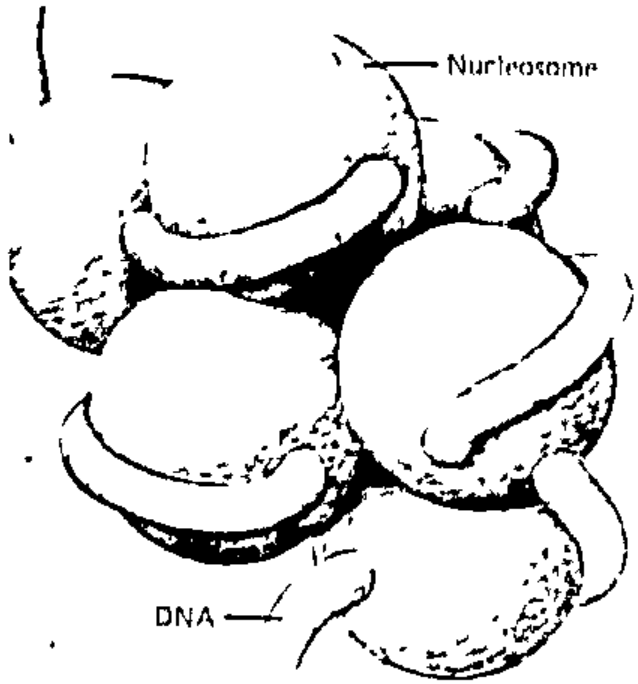


Fig 3 3 Model of the nucleosome package (From Vengerov et al , 1978 Reprinted with permission of VEB Georg Thieme, Leipzig)

ranging from many different biochemical approaches to different ways of cytologically analyzing the ultrastructure.

The classical approach in the study of chromosome structure is, of course, the cytological analysis. With the increase of resolution obtained by the discovery of the electron microscope it was hoped that the structure of the chromosome could be studied in detail. But because of the lack of good fixation methods and sufficient contrast, there still does not exist a convincing picture of the ultrastructure of chromosomes (see Chapter 1). Very fine fibrils, 2 nm to 4 nm in diameter, that appear like the double helix strands of the Watson-Crick DNA model have been observed in sectioned material. The dimensions of the chromatid pairs at the light microscope level are at the order of 200 nm. Since both phenomena—double helix and chromatids—are believed to have analogous linear arrangement of genes that are involved in crossing over, chiasma formation, and mutational events, the question of how the molecular structure can be built into the visible chromosomes arises. The present prevailing concept is that one long thread of DNA is arranged in the chromatid in some coiled or folded manner.

3.4.1 The Folded Fiber Model

The folded fiber model of chromosome ultrastructure is based on the method of whole mount electron microscopy described in Chapter 1. This model was developed by DuPraw (1965b, 1968) in order to integrate the large body of experimental genetic, cytological, and biochemical data with the new morphological discoveries. In this model each unreplicated **monad chromosome** or **unit chromatid** is loosely packed in irregularly transverse and longitudinally folded spirals of a single 20 nm to 50 nm elementary fiber which contains one extremely long single DNA double helix in supercoiled configuration held together by protein molecules. Replication of the chromosome occurs at several sites along the length of this fiber where DNA polymerase catalyzes DNA synthesis at fork configurations. The late replicating segments of the fiber at the centromere and elsewhere serve to hold together the sister chromatids (Fig. 3.4).

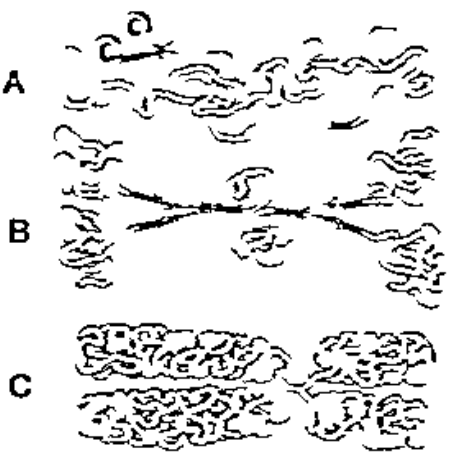


Fig. 3.4A–C. Diagram illustrating a folded fiber model of chromosome structure. (*A*) Each unreplicated chromosome (unit chromatid) is essentially a single 20–50 nm fiber that contains a DNA double helix in supercoiled configuration. (*B*) Replication of the chromosome occurs at several sites along the length of the fiber where DNA polymerase catalyzes DNA synthesis at fork configurations. (*C*) The late replicating segments of the fiber at the centromere and elsewhere serve to hold together the sister chromatids. (From DuPraw 1965b)

3.4.2 The Molecular Chromosome Model

DuPraw's folded fiber model is in line with the molecular model of chromosomes as formulated by Taylor et al. (1957), Taylor (1958, 1963), Freese (1958), and Schwarz (1960). The most important conclusion was that the chromosome is composed of only one DNA strand probably consisting of several molecules of DNA associated linearly. This model is based on the assumption that the chromosomal DNA follows a semiconservative mode of replication. The semiconservative mode was confirmed by tritiated thymidine labeling experiments that demonstrated that the DNA double helix separates into two separate polynucleotide strands as the hydrogen bonds between the nucleotide pairs break. As the two strands unwind, each synthesizes a new complementary copy of DNA nucleotides, leading to the formation of the two double-stranded DNA molecules, half derived from the parent molecule and half newly synthesized (Fig. 3.5).

One of the not so essential features of the molecular chromosome model is that the chromosome is composed of a number of subunit DNA helices linked end to end by a series of protein molecules (Fig. 3.6). Coiling and uncoiling of the chromosome may be explained by the interaction of such protein molecules with each other (Freese, 1958). Recent data seem to give strong evidence against the presence of such protein linkers. The integrity of DNA has been found to be sensitive only to deoxyribonuclease and not to ribonuclease or proteolytic enzymes.

3.4.3 The Multistranded Chromosome Model

One of the oldest and most seriously discussed arguments of chromosome fine structure has been the question of polynemy vs. uninemy. In spite of a recently prevailing consensus of uninemy or single-strandedness of DNA in the chromosome, some aspects of chromosome structure are still not readily explained by a

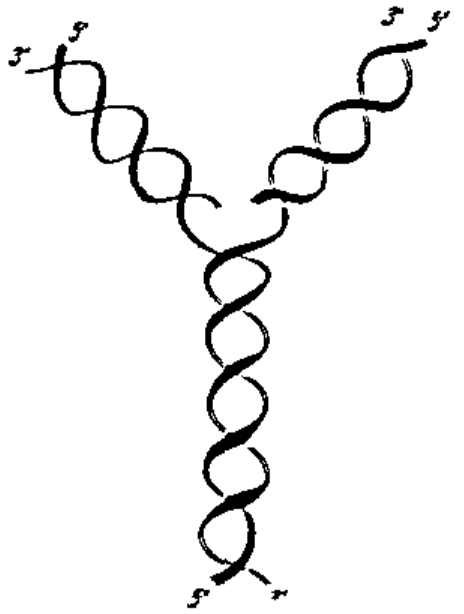


Fig. 3.5 Diagram showing the separating double helix at the beginning of replication and each of the strands producing a new strand. (Modified after Bolton, 1963)

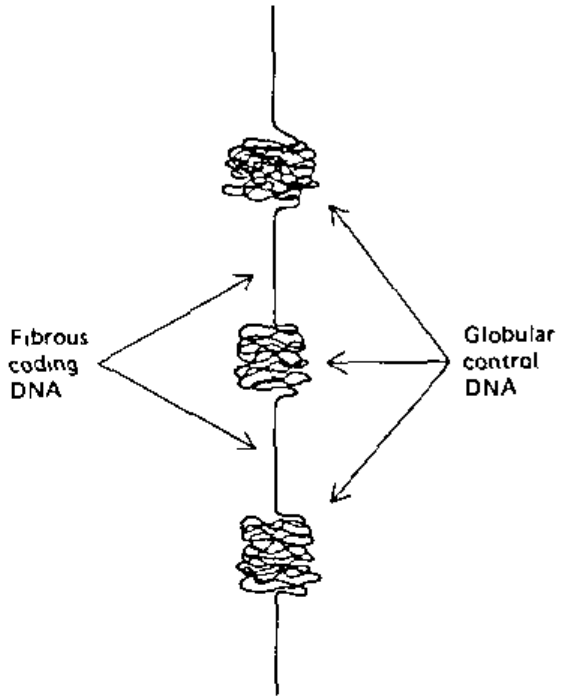


Fig 3 8 The "General Chromosome Model" according to Crick (1971) According to this model, the globular control DNA is in the bands and the fibrous coding DNA is in the interbands (Redrawn with permission of Macmillan Journals LTD London)

3 4 4 General Chromosome Model

In 1971 Crick proposed a general model for the chromosomes of higher organisms In contrast to the folded fiber model, this model is strongly based on findings in molecular genetics The model suggested that chromosomal DNA falls into two classes (1) globular control DNA in the bands and (2) fibrous coding DNA in interbands (Fig 3 8) This model also suggests that the DNA in a chromatid is a

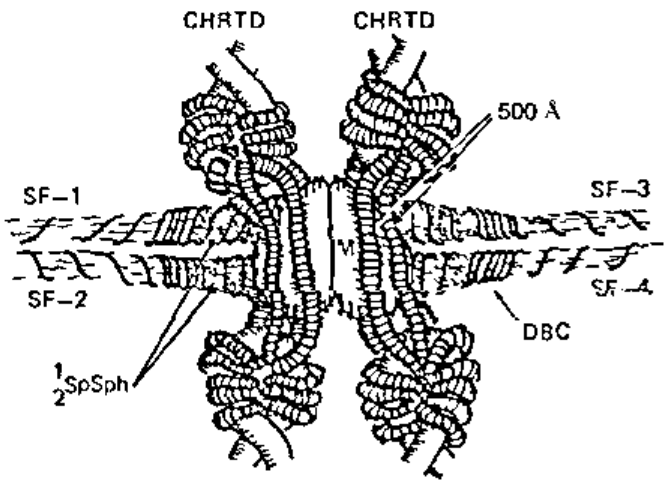


Fig 3 9 The Hoskins (1969) model of the centromere The chromatid arms (CHRTD) are extending upward and downward, the spindle fibres (SF-1, -2, -3, and -4) extend from the centromere to the left and to the right The chromonemata (50 nm) extend across the centromere from one arm of one chromatid to its other arm (M–matrix of the centromeres) (Courtesy of Dr Godfrey Hoskins, Hoskins Pathology Laboratories, Dallas, Texas Reprinted by permission of Caryologia Firenze Italy)

very long monomere (Prescott, 1970; Laird 1971) that probably runs continuously from one end of the chromatid to the other

This model has 3 basic features

1 The coding sequences of DNA are postulated to be mainly in the interbands (Vogel 1964)
2 The recognition sites needed for control purposes in higher organisms are mainly unpaired single stretches of double stranded DNA (Gierer 1966)
3 The forces and energy needed to unpair the recognition stretches of DNA are provided by the combination of DNA with chromosomal proteins—probably histones

3.5 Ultrastructure of the Centromere

A well-documented model of the fine structure of the centromere is based on electron microscopic analysis of Hoskins (1969) Hoskins used a method of micromanipulation by which he pulled the centromeres out of the cells for detailed study The model (Fig 3.9) shows two chromatids of a metaphase chromosome held together in the centromere regions by two hemispheric or valentine-shaped matrices (M) associated base to base The two arms of each chromatid are interconnected across the matrix by chromonemata (50 nm in diameter) that are continuous with the chromonemata of the chromatids This model seems to tend to a polyneme concept of the chromosome Also attached to the matrix are the spindle fiber bundles (SF) two to each matrix (a total of 4 to each centromere) In the area of attachment to the matrix, there is a swelling of the bundles which is called the spindle spherule

Part III
Function of Chromosomes

4.2 The Mechanism of Crossing Over

The mechanism of crossing over is still not understood and is closely related to the unsolved problem of chromosome ultrastructure. Consequently, several classical and newer hypotheses of crossing over exist. Only some will be discussed here.

4.2.1 The Partial Chiasmatype Theory

As mentioned in Chapter 1, Janssens in 1909 advanced the **partial chiasmatype theory**, which is now accepted as the most reasonable explanation of the relationship between cytologically observable chiasmata and experimentally demonstrated genetic crossing over. According to this theory, chiasmata are the direct result of crossing over and are formed exactly at the points where the breakage and exchange of nonsister chromatids occurs (Fig. 4.1). Crossing over occurs only between 2 of the 4 chromatids present at any given point, but three- and four-strand crossing over is possible in any given region.

4.2.2 The Belling Hypothesis

Belling's hypothesis (1931a, 1931b, 1933) correlates crossing over with the reproduction of new chromatids. The historical importance of this work was briefly mentioned in Chapter 1. Belling's hypothesis is the basis for some of the more recent theories and merits discussion in more detail. This hypothesis requires some kind of **relational coiling** (Section 6.3) between homologues at the time of chromatid reproduction (Fig. 4.2). The theory further postulates that new chromomeres are formed alongside their respective sister chromomeres without the formation of interconnecting fibers (Fig. 4.2A). The next step in this scheme is the formation of the connecting fibers between the newly synthesized chromomeres (Fig. 4.2B). During this process, sections of nonsister chromatids get intercon-

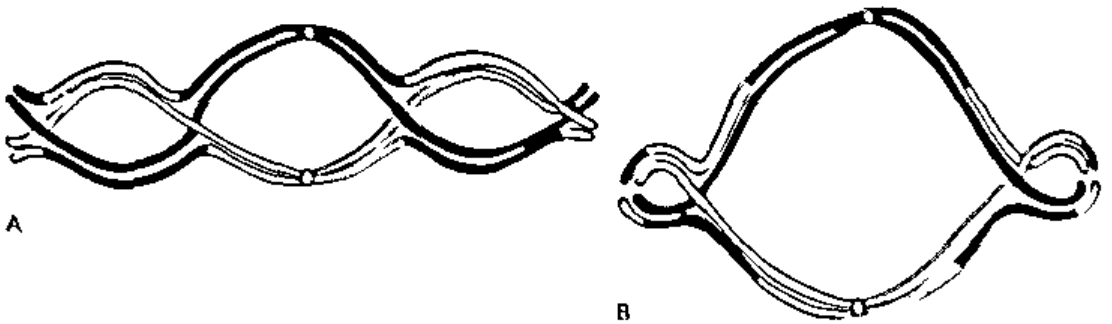


Fig. 4.1A and B. Schematic drawing of the cytological manifestation of crossing over in diplotene according to the partial chiasmatypy theory. (*A*) Four cytologically visible chiasmata occur at exactly the points where genetic crossing over has occurred in pachytene previously. Black chromatid segments may symbolize paternal chromosome origin and white ones maternal. (*B*) Advanced stage of chiasma terminalization in diakinesis. Chiasmata have started to move toward chromosome ends. Crossover points and chiasmata do not coincide anymore. Two chiasmata have become end chiasmata. (From Swanson 1957. After Darlington 1930. Redrawn by permission of Prentice-Hall, Englewood Cliffs, N.J.)

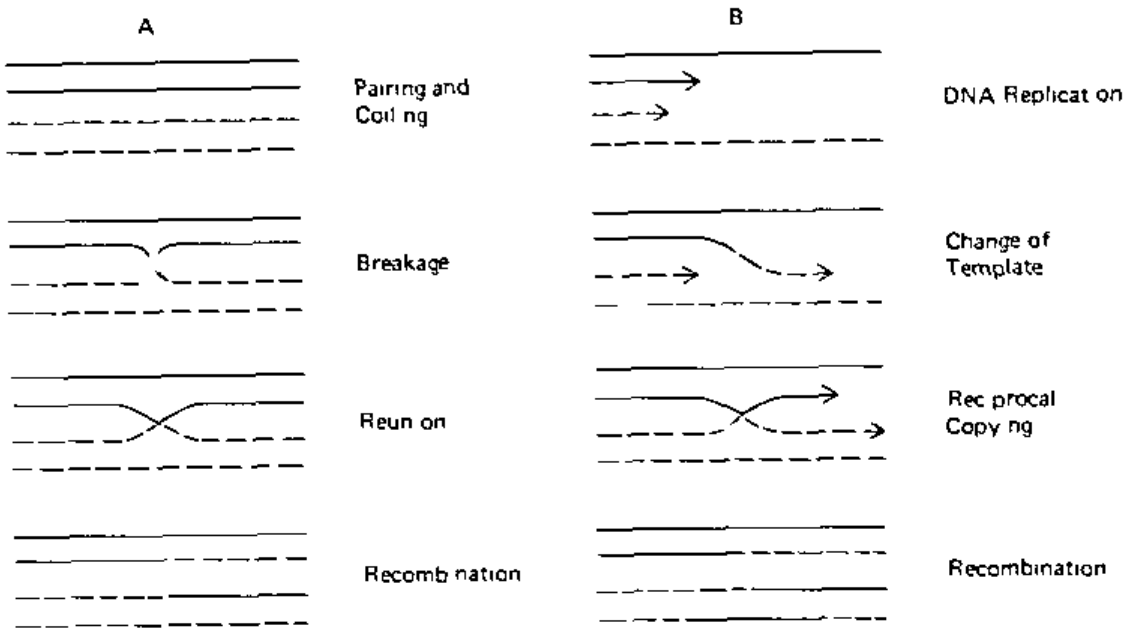


Fig 4 3A and B Schematic representation of the breakage-reunion (*A*) and copy choice (*B*) hypotheses of crossing over (From Hamerton 1971a Redrawn by permission of Academic Press New York)

two of the four chromatids present during crossing over are shown The figure (A) shows two non-sister chromatids each consisting of a DNA double helix The horizontal lines represent polynucleotide chains The arrows indicate the direction of the sugar-phosphate backbone and also delimit the extend of the **polaron.** The polaron is the unit of the chromosome by which it is subdivided in terms of **linkage points** where crossing over can be initiated The short vertical lines depict the hydrogen bonds between the bases of the complementary nucleotide chains The figure (B) also shows two opposite non sister chromatid nucleotide chains of opposite polarity breaking off enzymatically at one end of the polaron The broken nucleotide chains separate from their complementary chains over the main part of the polaron length New chains (broken lines in C) are synthesized along the polaron where the old ones were broken off After the new chains have been synthesized, they in turn also break off from their complementary nucleotide templates (D) The old and newly synthesized break products are now pairing up as **hybrid duplexes** to form new complementary DNA double helices (E) Any still existing gaps are now being filled with complementary nucleotide pieces (F) The old unpaired nucleotide chains are now breaking down by digestion and are thus eliminated (G, H) Crossing-over is now completed

4.3 The Cytological Basis of Crossing Over

Chromatid exchanges in meiosis are observable under the microscope by the formation of unmistakable structures between homologous chromosomes (homologues) called chiasmata (Fig 4 5) As can be seen clearly, a chiasma involves only one chromatid, but each of the two homologues are involved in its formation
The events taking place in meiosis, described later (Chapter 7), give us further

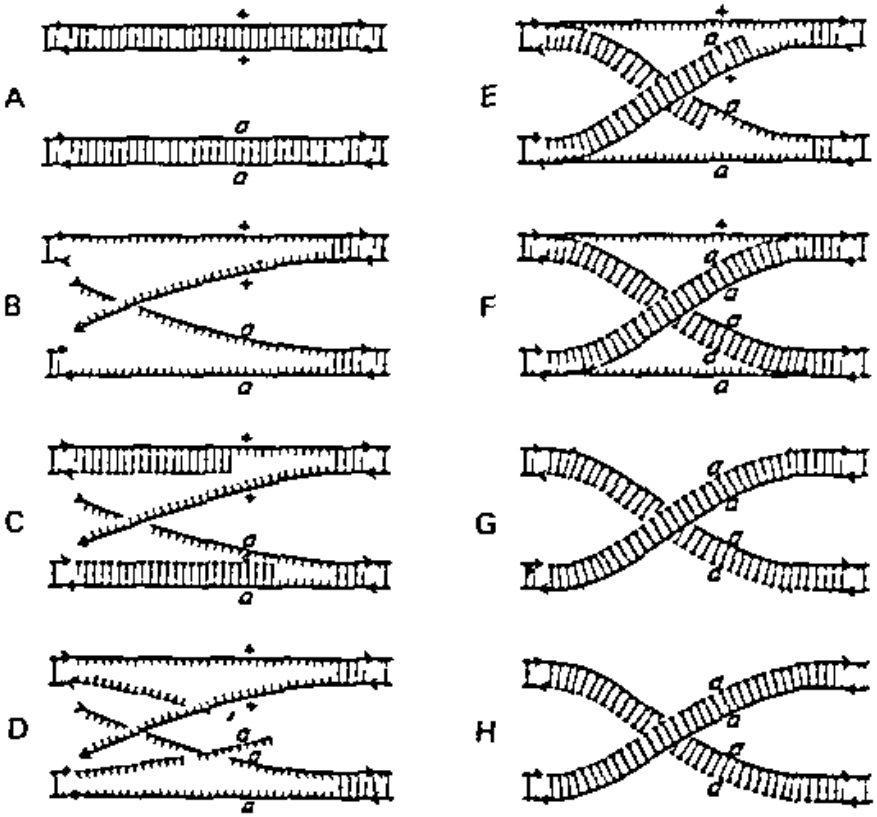


Fig 4 4 Diagram of the polaron hybrid DNA model of crossing over (From Rieger et al 1976 After Whitehouse 1965)

insight into the physical basis of crossing over It may be only mentioned here that homologous chromosomes are brought together in a snug union during early prophase and then separate during diplotene By this time the chromatid exchanges have taken place and at the exchange points the chiasmata begin to show up cytologically These then, are naturally the points of so-called "crossing over a phys

Fig 4.5 Photograph of a late diplotene bivalent in a spermatocyte of the Costa Rican plethodontid salamander *Oedipina poel i* (Courtesy of Dr James Kezer Department of Biology University of Oregon Eugene)

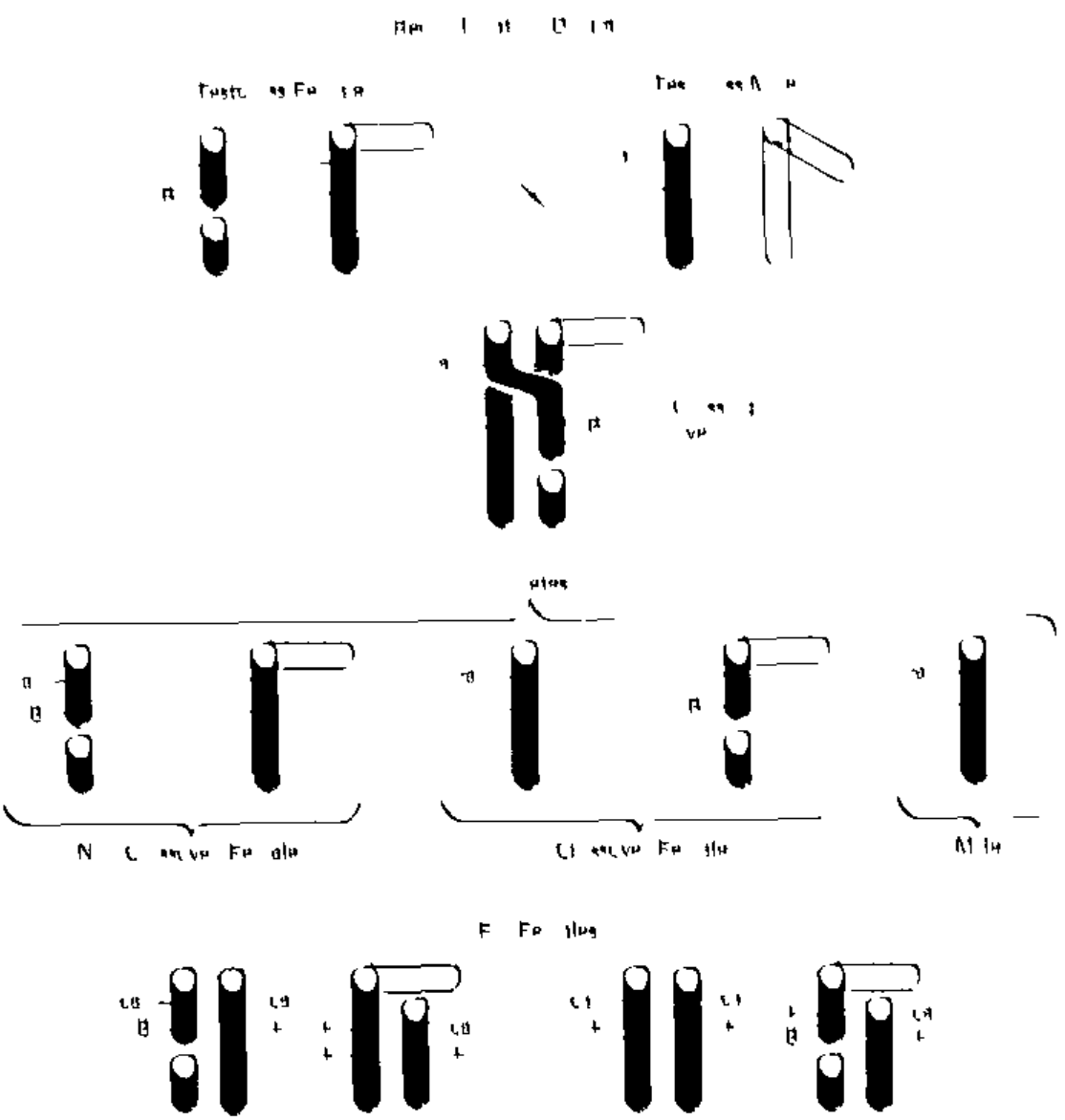
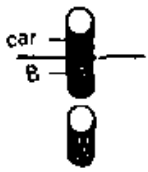


Fig. 4.6 Diagram modified from Stern (1941) to demonstrate that crossing over involves an exchange of chromatin between homologous chromosomes. Detail in text. (From Swanson, 1957. Drawn by permission of Prentice-Hall, Inc., Englewood Cliffs, New Jersey.)

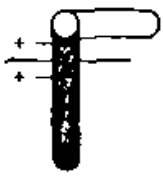
ical embodiment of a genetic term that originally designated a cytological or physical happening. Crossing over therefore represents the exchange of chromatid material between homologous chromosomes.

The actual demonstration of this exchange was accomplished by Stern (1931) and Creighton and McClintock (1931), as mentioned in Chapter 1. In Stern's experiment with *Drosophila*, a female carrier of two heteromorphic X chromosomes was used in a testcross. The two X chromosomes are explained in Fig. 4.6. The broken X chromosome carried the two marker genes, the recessive eye color *carnation* (*car*, 62.5) and the dominant eye shape *bar* (*B*, 57.0). The elongated X chromosome carried the two wild type genes (+). The testcross males carried an X chromosome with the two recessive genes (*car*, +). The female offspring of the testcross formed four classes: (1) *carnation* and *bar* with the parental broken X-chromosome; (2) normal eye color and shape with the parental elongated X chro



X–chromosome broken be ow the B locus Broken end is represented in the complement by be ng attached to chromosome IV (white circle = centromere)

Broken end



Elongated X–chromosome w th a large port on of the Y–chromosome (wh te) attached to the *car* end (horizontal l nes ind cate crossover location)

Broken X–chromosome with Y–chromosome portion attached to it resulting from cross ngover between car and B focus

Y–chromosome

Fig 4 6 (contd) Explanation of chromosomes used in Stern's (1931) experiment

mosome, (3) *carnation* and normal shape, a crossover type and (4) normal eye color and *Bar*-shaped eye with a crossover type broken and elongated chromosome Stern made a genetic and cytological analysis of 364 non crossover and crossover F_1 females, and in almost all of them there was exact agreement between the genetic and cytological data This demonstrated that genetic recombination was accompanied by a reciprocal exchange of chromatid material between the two homologous chromosomes

The number of chiasmata per pair of homologues is limited by the length of the chromosomes Adjacent crossovers or chiasmata do not occur independently A chiasma in a given chromosome region suppresses a chiasma in the adjacent region This has been called **chromosome interference** (Muller, 1916), **chiasma interference** (Mather, 1933), or **crossover interference** (Whitehouse, 1965) Interference increases with decreasing distance between successive genes and decreases with increasing distance

4.4 Locating Genes on Chromosomes and Genetic Mapping

One of the main functions of the chromosome is the block transfer of genes Cytogeneticists have been interested in first assigning genes to specific chromosomes and, if possible, locating their position on the chromosome

Several methods have been used to assign genes to their respective chromosome or linkage groups. Some of them will be only mentioned here and described in detail at the appropriate place in this book. Species with high numbers of chromosomes are difficult to work with for gene location. The discovery of monosomy and the establishment of monosomic series has helped in the assignment of genes to specific chromosomes or linkage groups. The first report of monosomics was in tobacco (*Nicotiana tabacum* 2n = 48) by Clausen and Goodspeed (1926) who at that time reported about plants with 47 chromosomes (48 − 1). Locating genes by the use of monosomics is discussed in Chapter 16.

The most extensive studies involving gene location have been carried out with trisomics (e.g., 2n + 1). In Chapter 1 the discovery of trisomics by Blakeslee (1921) was described. By the detection of trisomic ratios, genes can be associated with specific chromosomes. A more detailed description of trisomic gene analysis will also be given in Chapter 16. The use of translocations in mapping genes will be shown in Chapter 14.

Genetic mapping involves the assignment of genes to specific linkage groups and the determination of the relative distance of these genes to other known genes in that linkage group. This process assumes that genes are arranged in a linear order along the chromosome as first postulated by Morgan. The sequence of the genes on the chromosome can be determined from the three-point cross devised by Sturtevant (1913). The following example is taken from Swanson (1957). If one assumes that the correct sequential arrangement of three genes is *a b c*, then a testcross of the heterozygote $+ + +/a\,b\,c$ to the triple recessive $a\,b\,c/a\,b\,c$ would result in the following genotypes:

$+ + +/a\,b\,c$ $a\ b\ c/a\,b\,c$	noncrossover parental individuals
$+\ b\ c/a\,b\,c$ $a + +/a\,b\,c$	single crossover recombinants, class 1
$+ + c/a\,b\,c$ $a\ b + /a\,b\,c$	single crossover recombinants, class 2
$+\ b + /a\,b\,c$ $a + c/a\,b\,c$	double crossover individuals

As we know from incomplete linkage studies, the number of recombinant individuals is less than the number of parental combinations. Consequently, the number of the noncrossover parental individuals would be the highest. The frequencies in the two single crossover recombinant classes will depend on the distance between genes *a* and *b*, and between *b* and *c*. The number of individuals in the double crossover class will be the smallest. The double crossover class gives information concerning the linear arrangement of the genes along the chromosome. In the double crossover class (+*b*+ and *a*+*c*) gene *b* has shifted its position with its dominant allele with respect to genes *a* and *c*. The order of the three genes must therefore be *a b c*. An actual example of the three-point cross is shown in Chapter 14 (see Table 14.1).

Many such genetic data lead to the construction of genetic maps. Classical examples of such genetic maps are those of *Drosophila melanogaster* (Fig. 4.7) and of maize, *Zea mays* (Fig. 4.8). Each gene is shown on the genetic map as a point on

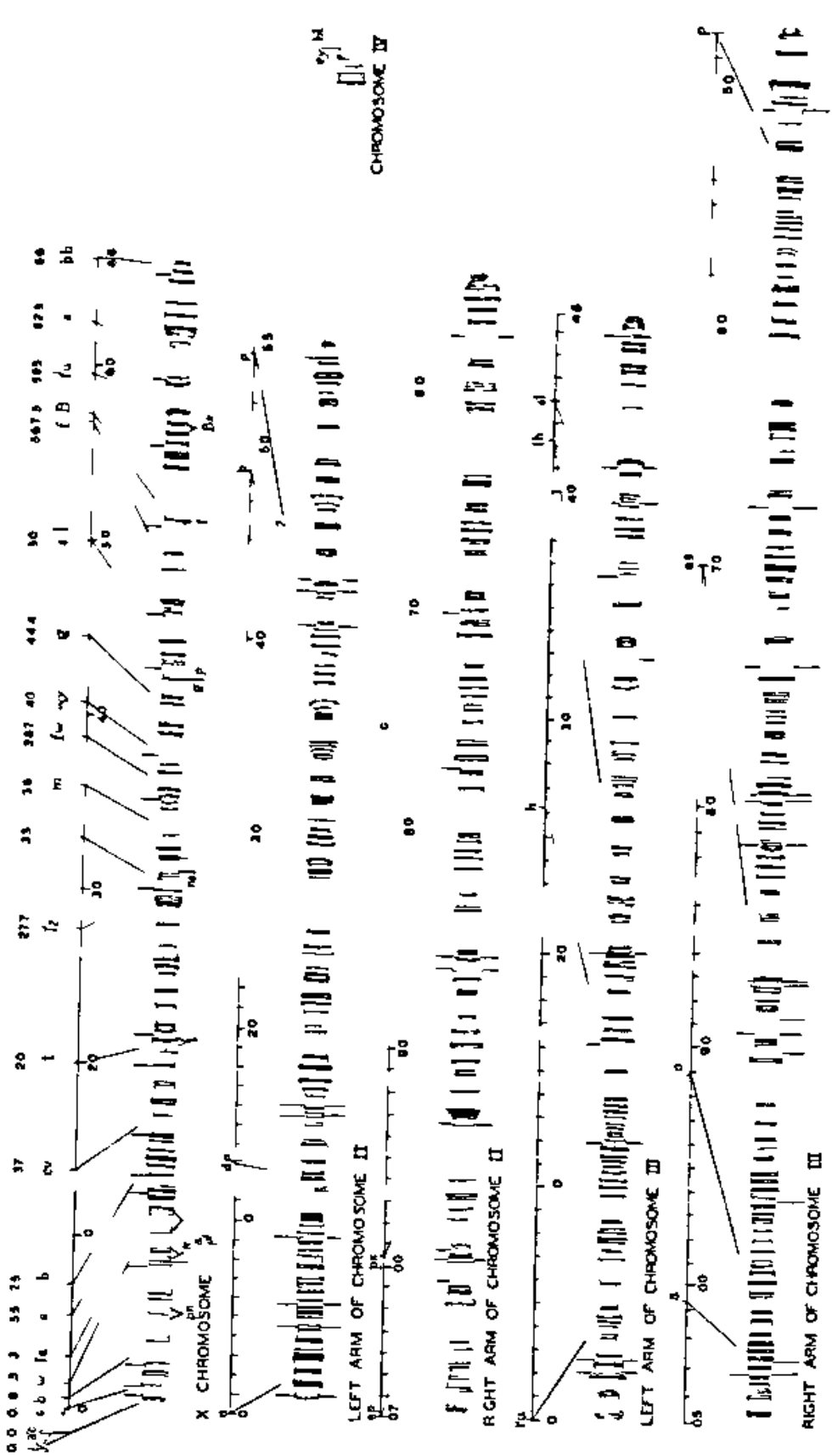


Fig 4 7 Reference map of the salivary chromosomes of *Drosophila melanogaster* (From Painter 1934b Reprinted by permission of the American Genetic Association Washington D C)

the linear chromosome The distance between any two genes is a function of the recombinant frequencies

Genetic maps do not reveal the *actual distance* of the genes because crossing over does not occur at the same frequency at different sections of the cytological or chromosome map In order to get actual distances one has to resort to **deletion mapping** or **cytogenetic mapping** This mapping seeks to determine the locus of a specific gene on the chromosome map Such locus may be detected for a specific arm for a fraction of such an arm or for a minute deleted segment of a chromosome To carry out such mapping a specific mutation can for instance be pinpointed to a deficiency (Chapter 11) in the corresponding chromosome

When an organism has a recessive nonlethal mutation in a chromosome and is heterozygous for a deficient segment on its homologous chromosome the recessive mutation will be expressed in the phenotype This phenomenon has been referred to as **pseudodominance**

The best results with deletion mapping have been achieved in the localization of genes on the giant salivary gland chromosomes of *Drosophila* These chromosomes are extended to such a size and have so much detail that small deletions can be traced with an excellent degree of accuracy

Mackensen in 1935 and Slizynska in 1938 used deletions to locate genes on the *Drosophila* chromosome map Slizynska s study is shown in Figure 4 9 The black areas on the diagram show the deficient regions of 14 different deficiency mutants all of which produce the *white-Notch* phenotype These areas are correlated to the numbering system of Bridges (1935) that divides parts of chromosomes with Arabic numbers uses capital letters for subdivisions and gives Arabic numbers to the bands within the subdivisions As an example band 3C7 is deleted in deletion mutants N8 Mohr 264-38 264-36 264 30 264 31 264-32, 264-33 264-37 264-39 264-2 and 264-19 (Fig 4 9) Bridges numbering system in turn is correlated to the bands on the chromosome map in Figure 4 9

In order to be able to appreciate the phenotypic expression of such mutated genes a picture of a fly with *notched wing* (*N*) is shown in Figure 4 10

Cytogenetic mapping also has been carried out in humans By 1977 over 110 gene loci had been assigned to specific human autosomes and about 100 more to the X-chromosome (McKusik and Ruddle 1977) The total number has since climbed to 347 (see Fig 4 11) The use of somatic cell hybridization for cytogenetic studies has been mentioned in Chapter 1 (Harris and Watkins 1965) The assignment of genes to specific chromosomes is a possible outcome of such studies

One such approach is the **synteny test** by which one can investigate if two genetic loci are linked to the same chromosome depending on their correlated loss or retention in hybrid cells The first such successful test was performed by Nabholtz et al (1969) who demonstrated that the loci for *HGPRT* (hypoxanthine guanine phosphoribosyltransferase) and *G6PD* (glucose 6-phosphate dehydrogenase) are both located on the X-chromosome

Another approach is the **assignment test** where the location of a particular gene on a specific human chromosome is demonstrated by the concordance between the presence or absence of this chromosome and a specific phenotype in many hybrid clones (McKusick and Ruddle 1977)

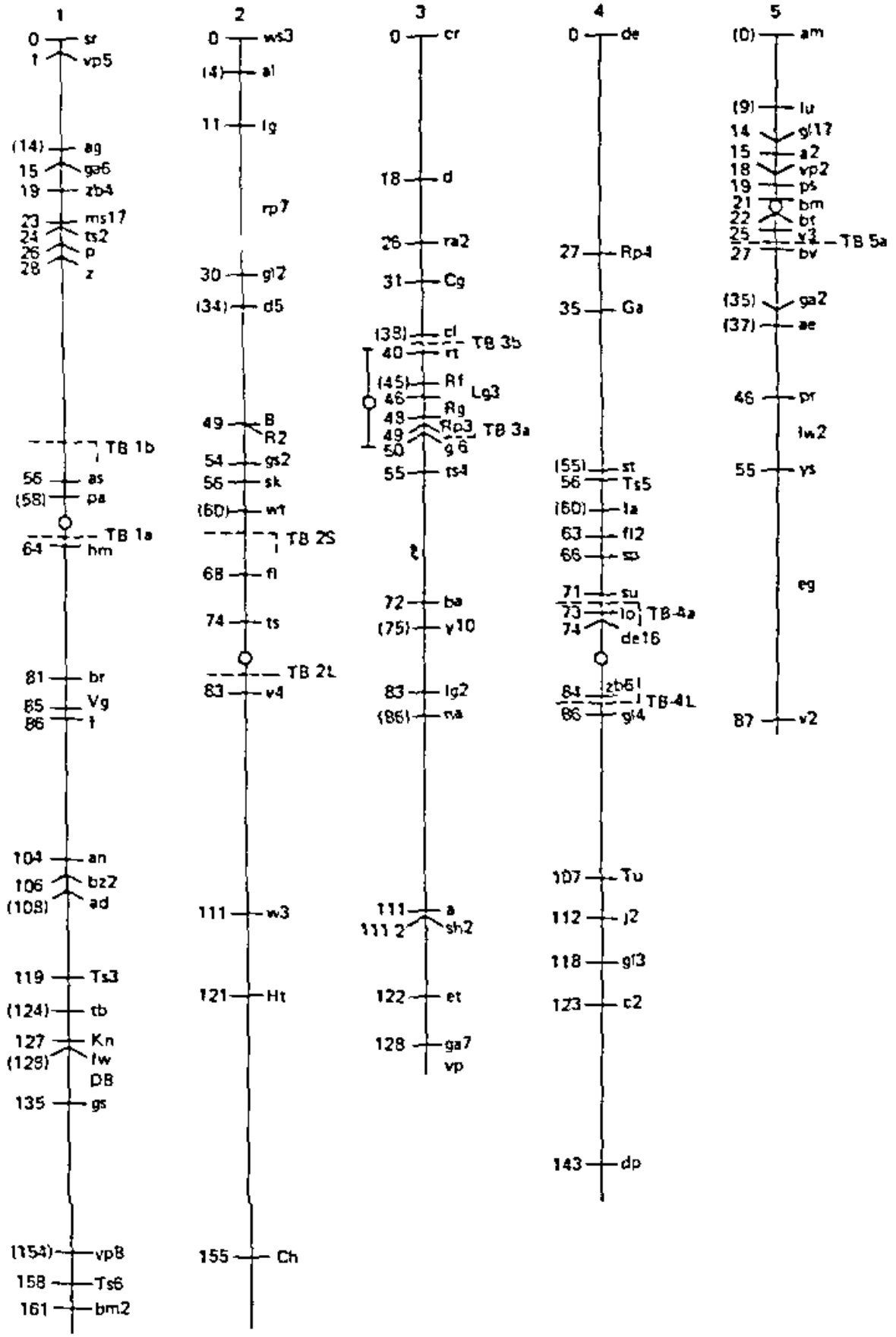
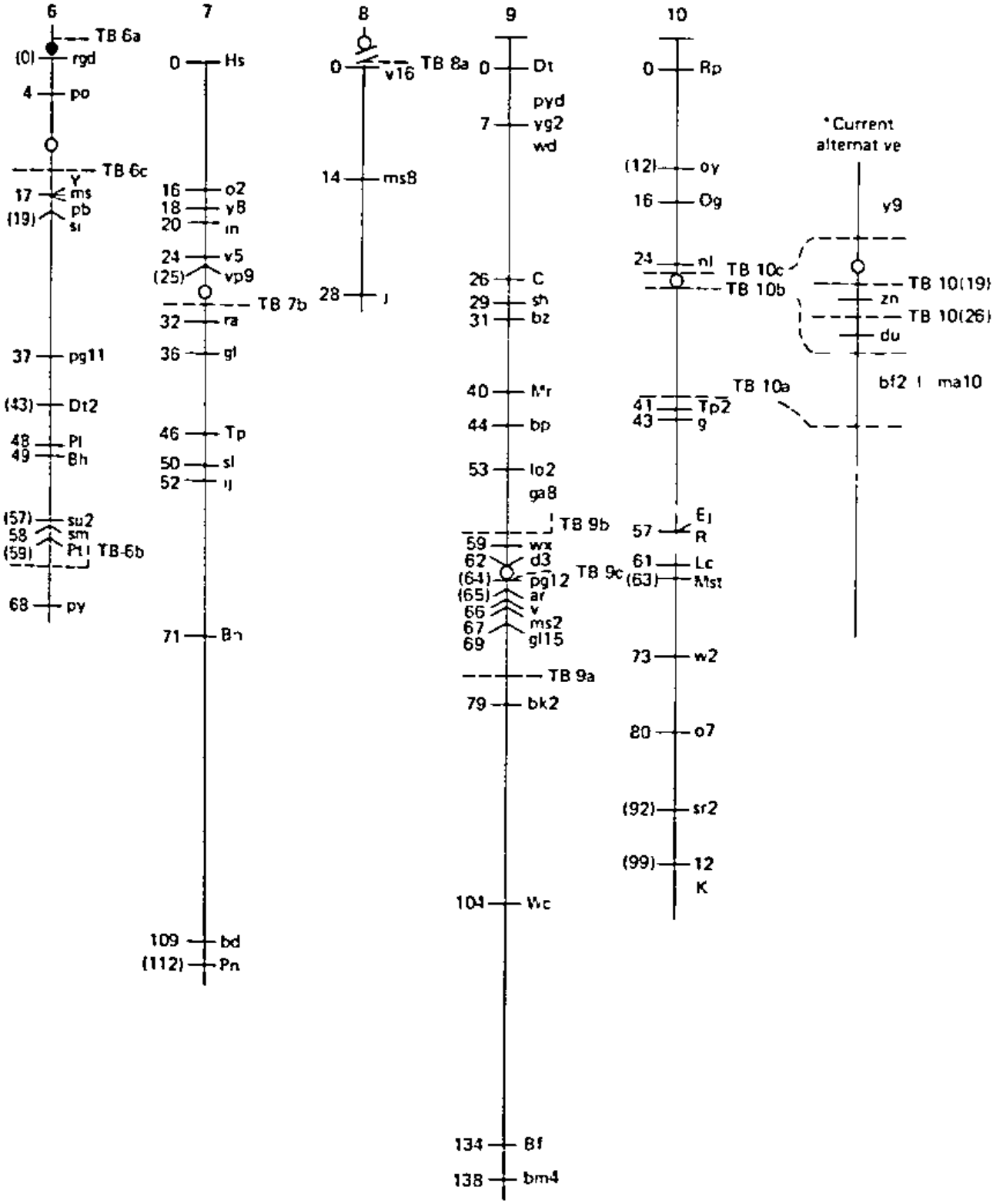


Fig 4.8 Genetic linkage map of maize (Revised from Neuffer et al., 1968 Reprinted from Maize Genetics Cooperation News Letter 51, 1977)



( ) Indicates probable pos tion based on insuff cient data

○ Ind cates centromere pos tion

● Ind cates organizer (NOR)

TB Indicates translocation involving A and B chromosomes with A break point at broken line or in d rection indicated TB 2S TB 2L and TB-4L are short designat ons for TB 2S 3L(6270) TB 2L 1S(4464) and TB-4L 9S(6222) from Rakha and Robertson (1970) TB 10(19) and TB 10(26) are as designated by Lin (1974)

* Lin (1974) Beckett (Personal communication)

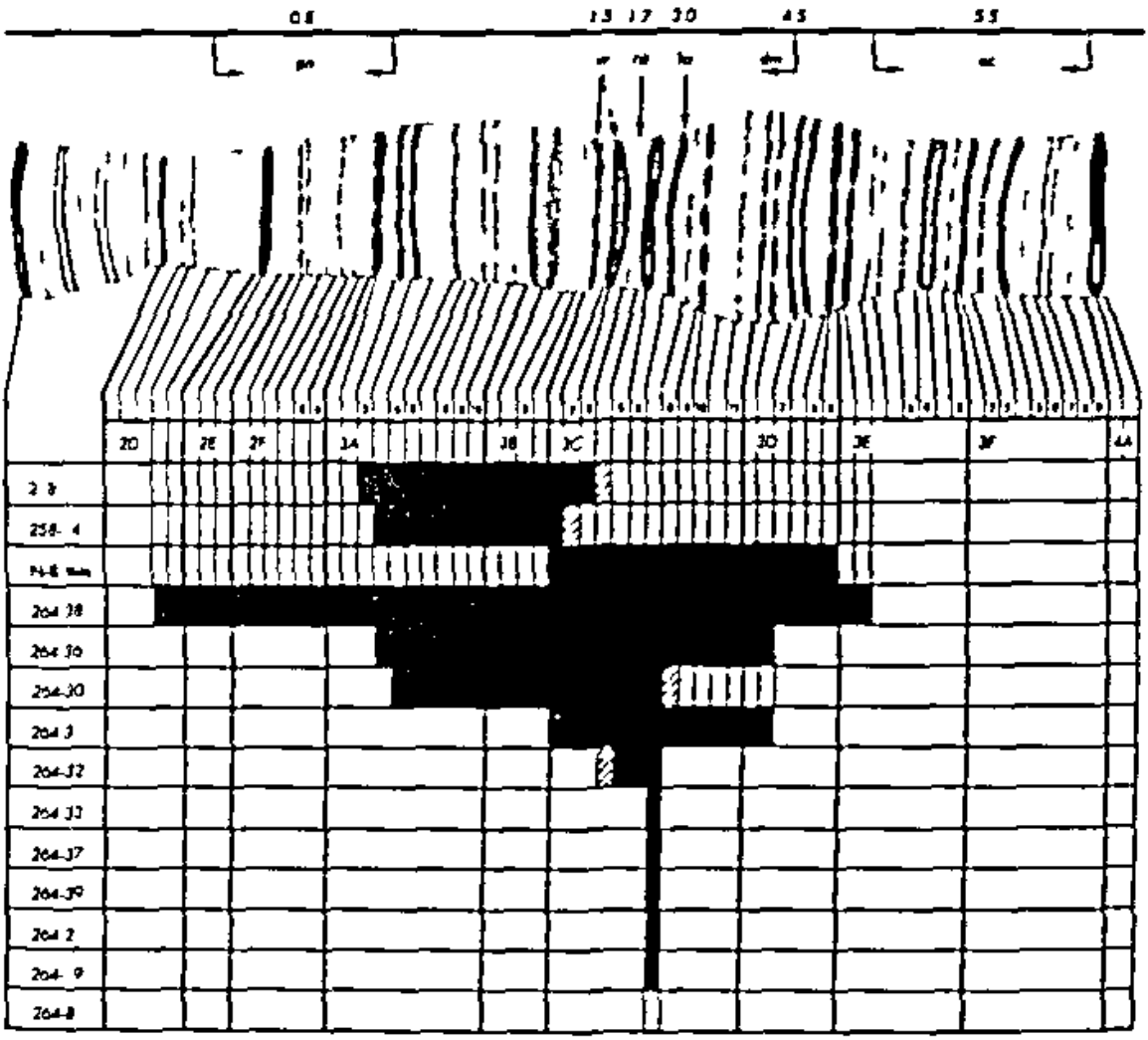


Fig 4 9 Deletions in the left end of the X-chromosome that have been used to locate genes on the *Drosophila* chromosome map Black segments shown below indicate bands removed by each deletion (From Slizynska 1938 Redrawn by permission of Prentice Hall Inc Englewood Cliffs, N J)

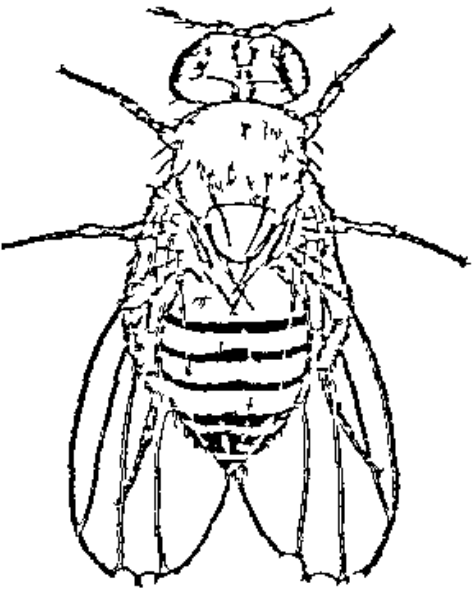


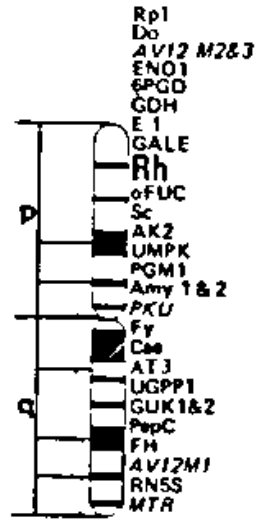
Fig 4 10 Drosophila fly with *notched* wing (*N*°) (From Mohr, 1924)

Regional mapping is used to assign a human gene locus to a specific chromosome segment. Here a human cell with a particular chromosomal rearrangement is used as parent in a cell hybrid. Such rearrangement would be a translocation. The translocation breakpoint divides the chromosome under investigation into two independent segregating units that can be separately studied in the assignment test. A **human mutant cell bank** (Coriell 1975) has helped to store, distribute and analyze such chromosomal translocations and deletions. The first regional mapping assignment through the use of a chromosomal translocation was that of *G6PD, PGD* (6-phosphogluconate kinase) and *ESD* to the long arm of the X chromosome (Rucknagel and Raddle, 1967).

In situ hybridization of RNA and DNA has been used as an approach to gene mapping in humans. Such nucleic acid hybridization can be performed in the test tube (Spiegelman, Chapter 11) as well as inside the cell. Here dissociated and denatured DNA is set in place inside the cell nucleus. It can be subjected to a hybridization with isolated RNA. If the RNA is radioactively labeled, the chromosomal location of the specific genes from which the RNA is normally transcribed can be actually determined under the microscope (Henderson et al., 1972) as well as Gusella and Pearson (1974) used this method to localize genes for 18S and 28S ribosomal RNA on the short arms of the satellite chromosomes (*D* 14, 15 and 21).

Deletion mapping, discussed in Chapter 11, has also been applied in human chromosome mapping. The first assignment by deletion mapping of a human gene not previously located by any other method was that of *ACP 1* (acid phosphatase) to the distal end of the short arm of chromosome 2 (Fergusen-Smith et al., 1973).

Duplication mapping, like deletion mapping is a gene dosage method. A person trisomic for part or all of a chromosome has about 50 percent more of a particular gene product. The location of five human genes has been confirmed by duplication mapping. These are the assignment of *aminoacyl-tRNA synthetase* (Tan et al., 1974) and *SOD-1* (superoxide dismutase-1) (Siniscalco et al., 1973) to chromosome 21, *GALT* (Galactose 1-phosphate uridyl transferase) to chromosome 9 (Kalderon and Tedesco, 1973), *ACP 3* (red cell acid phosphatase 3) to chromosome 2 (Weemaes et al., 1973) and *glutathione reductase* to chromosome 8 (Chapelle et al., 1967). A genetic map of human chromosomes is shown in Figure 4.10.



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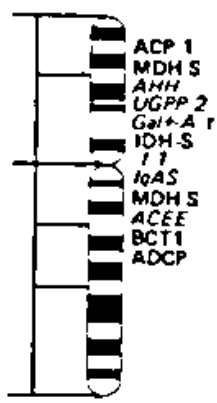

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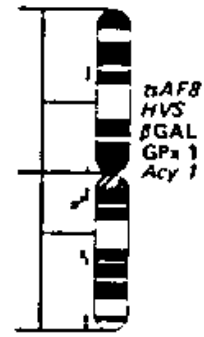

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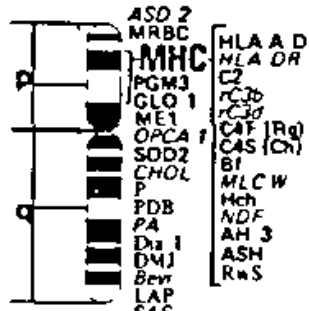

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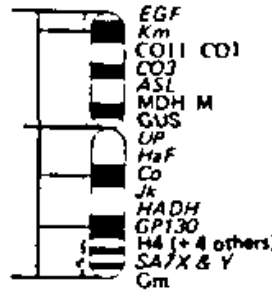

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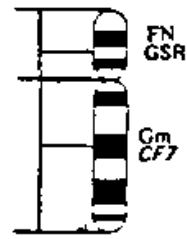

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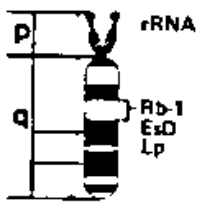

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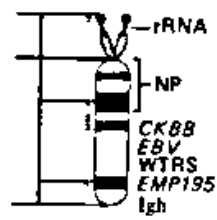

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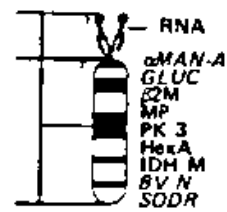

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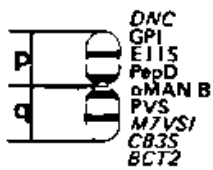

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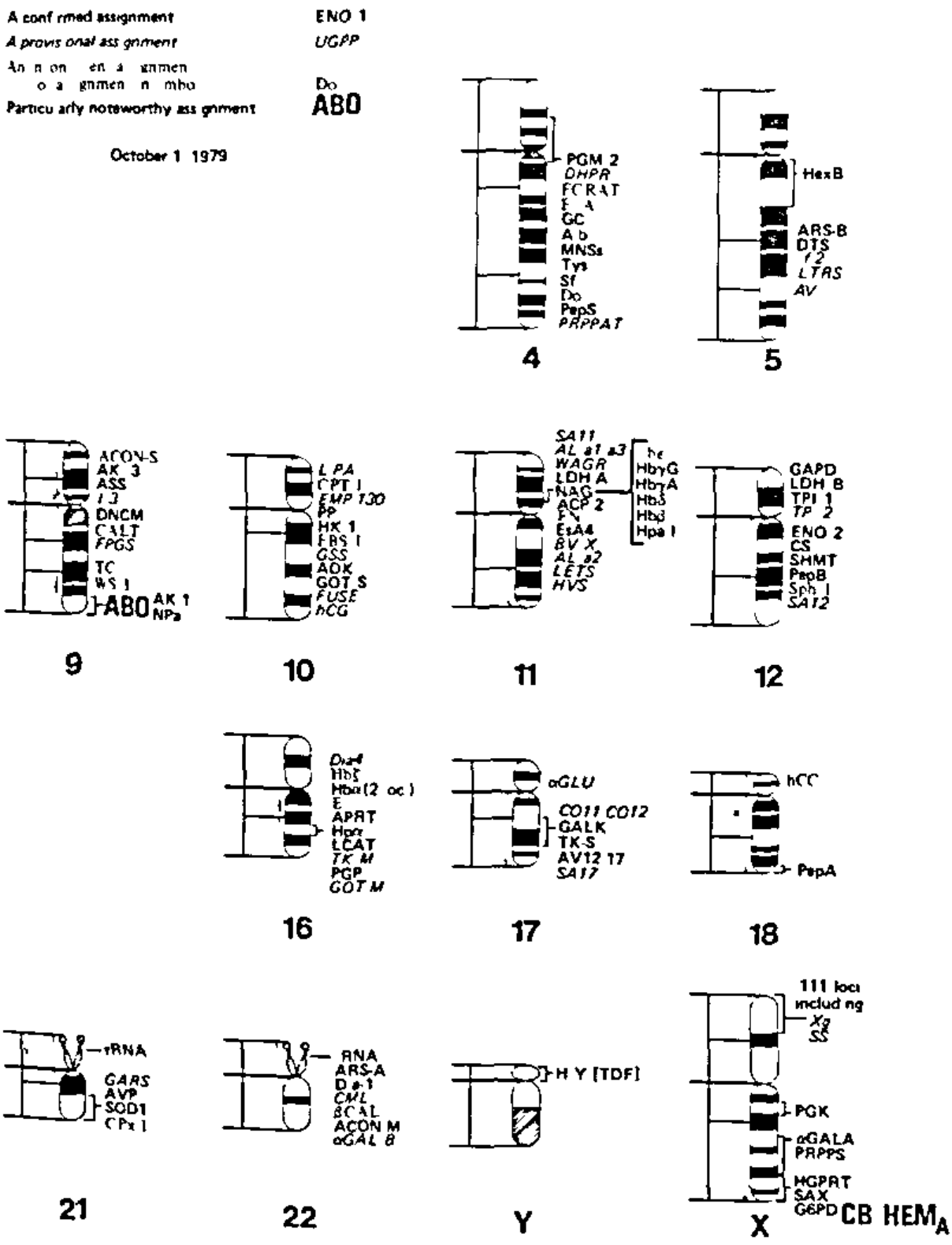


Fig 4 11 Genetic map of the human chromosomes (Courtesy of Dr V A McKusick Department of Medicine Johns Hopkins Hospital Baltimore Maryland)

Chapter 5
Function of Sex-Chromosomes

By now it may be obvious that the author emphasizes the historical importance of the discoveries that made cytogenetics the science it is today. That is why the first chapter was written in such detail and the student is often referred back to it in order to freshen his recollection. The first studies of chromosomes that determine sex were undertaken at the end of the last century. As mentioned in Chapter 1, Henking in 1891 for the first time described what is now known as the X chromosome. Half of the sperm of the insect *Pyrrhocoris apterus* received this chromosome and half did not. This system is now known as the X O system. A much more common system is the X Y system, which will be discussed first.

5.1 The X-Y System

The X Y system is a basic form of sex determination in animals and in some plants. This involves a structural difference between the sex chromosomes that can be observed cytologically. A homologous pair of sex chromosomes may be unequal in size and shape in one sex (**heteromorphic**) but equal in size in the other (**homomorphic**). The sex that harbors the heteromorphic sex chromosome pair was called by Wilson (1911) the **heterogametic sex** because during meiosis it produces two types of gametes, one male determining and one female determining. He called the sex with the homomorphic chromosome pair the **homogametic sex** since it produces only one type of gametes. In most vertebrate and in many insect species the heterogametic sex (XY) is the male, and the homogametic sex (XX) is the female. However, in birds, in some moths, and in fishes, amphibians, and reptiles, for instance, the opposite relation is true in that the male is the homogametic sex (XX). The homologies of the X and Y chromosomes can vary from species to species. The sex chromosomes (X and Y) may have a long **homologous region** and a very short **differential region**, or a short homologous region and a long differential region. In some species such regions have been mapped according to their size. In *Melandrium album*, for instance, the homologous or pairing region is very short, proportionally speaking. In Fig. 5.1 a diagram of the X and Y chromosomes of *Melandrium* is presented.

As shown in Fig. 5.1, there are male and female supressor regions as well as male and female promoting regions on the sex chromosomes, depending on the role of

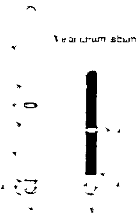


Fig. 5.1 The sex chromosomes of *Melandrium album*
I—female suppressor region
(when absent leading to bisexual development)
II—essential male promoting region
(when absent leading to female development)
III—essential male fertility region, secondary sex character
(when absent, anther abortion)
IV—pairing region of both X and Y
(length arbitrary)
V—differential portion of X, has the sex genes for femaleness
(From Westergaard, 1948. Reprinted by permission of the Mendelian Society, Lund, Sweden)

these chromosomes in sex determination. Several theories on sex determination have been developed by different investigators. Naturally, these theories depended greatly on the organisms that were investigated by these different researchers.

5.1.1 Bridges' Balance Theory

In 1932 Bridges developed the **balance theory**. He studied *Drosophila* which normally has 2n = 8 chromosomes, but for his studies he used individuals that had 3n = 12 chromosomes called **triploids** (Chapter 16). Bridges crossed these individuals with normal diploid males and recovered several different chromosome combinations in the offspring. Depending on the balance between the X chromosomes and the autosomes (A), Bridges observed different degrees of maleness or femaleness. He expressed this balance in an X:A ratio between X chromosomes and autosomes. The Y chromosome did not seem to have any effect on sex determination in *Drosophila*. The results of this study are shown in Table 5.1. As seen

Table 5.1 Chromosome constitution and sex in *Drosophila* (A = one set of autosomes)

Chromosome constitution	Sex	X:A ratio
2A XXX	Superfemale	1.5
2A XX 2A XXY 3A XXX 4A XXXX	Female	1.0
3A XX 3A XXY	Intersex	0.67
4A XXX	Intersex	0.75
2A X 2A XY 2A XYY 4A XX	Male	0.50
3A X	Supermale	0.33

in a normal female, the balance between two sets of autosomes and two X chromosomes produces femaleness (X/A = 1), while in a normal male, the autosomes outweigh the X chromosomes (X/A = 0.5) resulting in maleness. The X chromosomes seem to influence female development, while the autosomes seem to influence male development. Other imbalances produce superfemales, supermales, and intersexes.

5.1.2 Goldschmidt's Theory

Goldschmidt's work (1934) was carried out on the gypsy moth, *Lymantria dispar*. In this organism the male is homogametic (XX), and the female is heterogametic (XY). The gypsy moth was chosen for these studies because intersexes (gynandromorphs) are common in this species. Goldschmidt concluded that in *Lymantria* the sex was determined by the relative strength or balance of a female determining factor (F), which is inherited from the mother, and male determining factors (M), which are located on the X chromosomes. At different times Goldschmidt thought that the F factor was either carried on the Y chromosome or in the cytoplasm. Figure 5.2 depicts the second contention. In this scheme the female formula is F/M (F>M), and the male formula is F/MM (MM>F). F- and M determiners differed in potency in different races, their relative strengths were approximately the same in every race. There were strong and weak F's and strong and weak M's. Intersexes occurred regularly among the offspring of crosses between different geographical races. If a female with strong F and weak M was crossed with races that had M's of different strength, the XX offspring, even though being genetically males (chromosomal sex), were not necessarily phenotypical males. If the introduced M-determiners were strong, the offspring was male, if the introduced M determiners were weak, the phenotypical expression of the offspring was female. Intersexes occurred when male- and female determiners were in balance. Goldschmidt concluded further that the different strengths of M depended on 13 genes, the different strengths of F on 8 genes or cytoplasmic dosage factors. Goldschmidt (1915, 1916, 1920, 1922, 1923, 1929, 1934) and his coworkers also

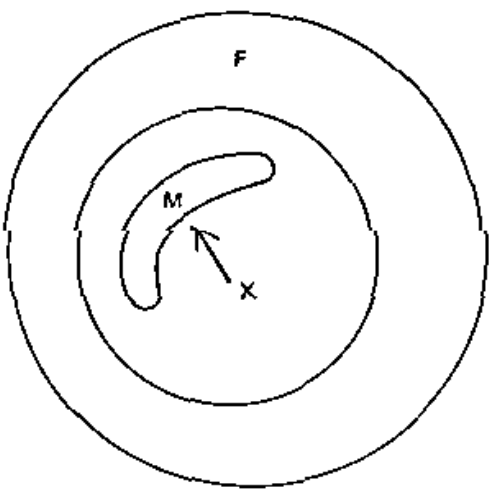


Fig. 5.2 Sex expression in *Lymantria dispar* according to Goldschmidt (1934). The female-determining factor is carried in the cytoplasm, the male determining factor in the X chromosome

proposed a mechanism by which these sex factors influenced the development of the sex phenotype He called this mechanism the **time law**. He assumed that the intersexes begin their development as females or as males (chromosomal sex) and develop as such to a certain point, called the **turning point**, after which they developed into the opposite sex The degree of intersexuality is determined by the timing of the switch-over in differentiation

5 1 3 Pipkin's Theory

Pipkin (1940 1942, 1947, 1960) concluded from her work that the sex in *Drosophila melanogaster* was decided by a balance between male-determining factors in the second and third and female-determining factors in the X chromosomes (Fig 5 3) Her work was based on a method developed by Dobzhansky and Schultz (1934) that added or subtracted broken pieces of an X to the normal 2X of triploid intersexes (see Table 5 1) The feminizing effect of the X portion could be measured by the degree of intersexuality in the flies No single female sex genes could be located through these very thorough studies The feminizing effect of the extra X sections was proportional to the size of these sections It was concluded that many female sex genes were spread over the X chromosome The conclusion that the male-determining factors were located in the third chromosome came from the following process of elimination Bridges (1922) originally assumed that the male-determiners must be carried in the autosomes Obviously, the fourth chromosome must be disregarded as a carrier because haplo-IV and triplo-IV *Drosophila* individuals do not affect the intersexes Pipkin (1947) carefully checked the second chromosome with the translocation and triploid method and did not find any sex-determiners in it She likewise did not find sex-determiners if the second chromosome only was involved (Pipkin, 1960) Her suggestion was that both the second and the third chromosomes are responsible for the shift toward maleness found in ordinary 2x3A triploid intersexes (see Table 5 1)

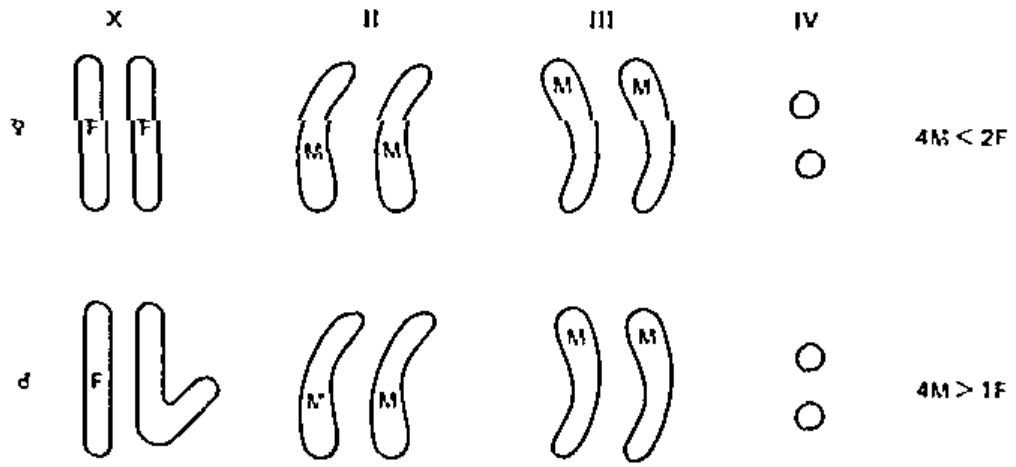


Fig 5 3 Pipkin's (1947) theory on sex determination in *Drosophila* Explanation in the text

5 2 The Function of the Y Chromosome

The function of the Y chromosome varies according to the organism An excellent review on this subject is published by Dronamraju (1965) This chromosome may vary from bearing some functional units of great importance to being completely lost as in the already mentioned X O system The Y chromosome mostly has a high proportion of **heterochromatin**, which is generally considered as having a high degree of genic inertness Historically, the Y chromosome was considered to contain degenerate genes or no genes at all (Muller, 1914a, 1914b) This idea was based on some of the early discoveries In *Drosophila* flies without Y chromosomes (XO) were viable but flies without X chromosomes (YO, YY) were inviable The Y chromosome consequently was not necessary for survival Females with additional Y chromosomes (XXY) were indistinguishable from normal flies (Muller, 1914a, 1914b) Males with two Y's (XYY) also did not show any different morphology As shown in Table 5 1, the Y did not seem to have any appreciable effect on the sex expression of *Drosophila*

The sudden discovery in 1959 (Ford et al , Jacobs and Strong) that the Y in man is strongly male determining drastically changed the earlier conclusions Later it was found that even XXXXY individuals and mosaics of the type XXXY/XXXXY/XXXXXY are phenotypically male in man (Anders et al., 1960) Work with mice, cats and other mammals also indicated that the Y is male-determining in these animals

In the flowering plant wild campion of the pink family, *Melandrium dioicum* as in man and mammals, the Y chromosome is also strongly male-determining This is the only plant species in which the function of the Y chromosome has been intensively investigated (Warmke, 1946, Westergaard, 1958) It is interesting that this study predates the findings in man by 14 years, but it did not have the same impact as the discovery in man In *Melandrium* the XYY, XXY, and XXXY types are all male, but the XXXXY is hermaphrodite The Y chromosome is larger than the X chromosome in this species (Fig 5 1) In many species the Y chromosome is much smaller than the X, which also has been used as an argument of its relative inertness

Very few genes have been located on the Y chromosome They are referred to as **holandric genes** (Enriques 1922) The first Y-linked gene in any species was the one for a black pigment spot in the fish, *Lebistes reticulatus* (Schmidt 1920) Only two Y linked characteristics are presently listed for the human gene map (McKusick and Ruddle, 1977) They are the *histocompatibility gene* (*H-Y*) and the *testis determining factor* (*TDF*) From studies of chromosome aberrations it was concluded that these two genes may be at the same locus on the short arm of chromosome Y close to the centromere (Wachtel et al , 1976) *H-Y* regulates immunological properties of histocompatibility antigens Histocompatibility antigens determined by the Y chromosome have also been reported for mice rats, and guinea pigs (Wachtel et al , 1974) Other characteristics were located on the human Y chromosome at various times but firm evidence is lacking

5.3 Dosage Compensation

The term **dosage compensation** was coined by Muller et al (1931) in order to account for the fact that in *Drosophila* there must exist mechanisms that equalize the effective dosage of sex linked genes in the male (XY XO) and female (XX) organisms For instance, in *Drosophila* the X chromosome carries many sex linked genes that are not responsible for sex expression Such genes do not have corresponding alleles on the Y chromosome and are consequently present in a **hemizygous** condition in the males However, males and females are morphologically and physiologically so similar in expression that it seems that one gene dosage is as effective as two We will see later that the effect of the dosage has an appreciable effect on gene expression in individuals that have missing or additional chromosomes (Chapter 17) In man even the monosomic or hemizygous condition of the smallest chromosome (one fourth of the size of the X) is lethal The fact that the hemizygous (XY, XO) and disomic (XX) conditions of the X chromosome have similar phenotypic expression has been explained with some kind of dosage compensation mechanism

In *Drosophila* dosage compensation has been explained to be the result of the action of modifier genes, so-called **dosage compensation genes,** on the X chromosome that cancel the effect of different doses of a given gene (Muller, 1947) The dosage compensation mechanism of *Drosophila* appears to operate by forcing a given X-linked gene in the XY-male to work harder, while restraining the activity of the same X-linked gene on each of the two X's of the female (Ohno, 1967)

5.3 1 The Single Active X Hypothesis

In man and mammals another mechanism seems to provide for the inactivation of the second X chromosome as a means of dosage compensation This mechanism is called the **single active X hypothesis** or **Lyon hypothesis** (Lyon, 1961, 1962a, 1962b, 1963, 1970, 1971, 1972) mentioned in Chapter 1 The Lyon hypothesis makes the following conclusions

1 In XY-males, the single X chromosome is active in all cells, while in each cell of the female (XX) one of the two X chromosomes becomes inactivated
2 Paternal and maternal X chromosomes have an equal chance of being inactivated
3 Inactivation occurs early in the life of the female embryo This implies that in XX organisms both X chromosomes are euchromatic and active in RNA synthesis during early embryonic development
4 Once it has been decided which X chromosome is inactivated in a cell, the same X chromosome will always be inactivated in the descendants of that cell
5 The inactive X chromosome becomes heterochromatinized and forms the sex chromatin found in interphase, which is believed to be the late replicating X chromosome (Ohno and Hauschka, 1960)

Lyon's hypothesis is derived from her studies of the X-linked coat color genes in mice These dominant genes produce different phenotypes in males and females If males, for instance, carry the gene for mottled (*Mo*), the mice will have a uniform coat color (*Mo/Y*) If females are heterozygous for mottled (*Mo/+*), they have a

Fig 5 4 A female mouse heterozygous for the X linked gene *dappled* ($Mo^{dp}/+$) having a variegated coat with particles of mutant and wildtype color (After Lyon 1966 Reprinted by permission of Paul Elek Limited London)

variegated coat with patches of mutant or wild type color The same pattern is produced in females carrying the gene for dappled (Mo^{dp}) (Fig 5 4) The pigmented wild type patches (+) descended from cells in which the X chromosome carrying the mutant gene (*Mo*) was inactivated The mutant patches (*Mo*) originated from cells in which the X chromosome carrying the wild type gene (+) was inactivated

Application of the Lyon hypothesis to human beings has come from the study of cultured skin fibroblasts (Beutler et al 1962 Davidson et al 1963) They found electrophoretic variants of glucose 6 phosphate dehydrogenase (*G6PD*) Clones from *G6PD* heterozygous females were 50% normal and 50% deficient

5 3 2 Sex Chromatin and Drumsticks

The discovery of sex chromatin or **Barr bodies** by Barr and Bertram (1949) in cats is closely related to the phenomenon of dosage compensation Barr s discovery was based on some earlier findings During the first decade of this century Montgomery (1904 1906) discovered the **heteropycnotic** behavior of the X chromosome in the male germ line of the hemipteran insect *Pyrrhocoris* Heteropycnotic chromosomes are those that are out of phase (**allocycly**) if they are compared with the **coiling cycle** in which the rest of the chromosomes of the set are engaged They are also out of phase in respect to their staining properties **Positive heteropycnosis** is the condition of the chromosome when it is densely coiled **negative heteropycnosis** is the condition when it is less spiralized These terms correspond

to the expressions **heterochromatin** and **euchromatin,** mentioned in Chapters 1 and 2 that designate the staining properties of chromosomes Densely coiled pos itively heteropycnotic material is considered to be heterochromatic or darkly staining and less spiralized Negatively heteropycnotic material is considered to be euchromatin it has normal and less darkly staining properties in interphase and prophase

In order to understand the nature of sex chromatin one should distinguish between **facultative** and **constitutive** heterochromatin (Brown 1966) Facultative heterochromatin is euchromatin that can be heterochromatinized during the cell cycle Constitutive heterochromatin is always heterochromatinized and it is the usual form It is the chromatin that is found in the centromere regions near the telomeres in the satellites and in the nucleolus organizer region Constitutive het

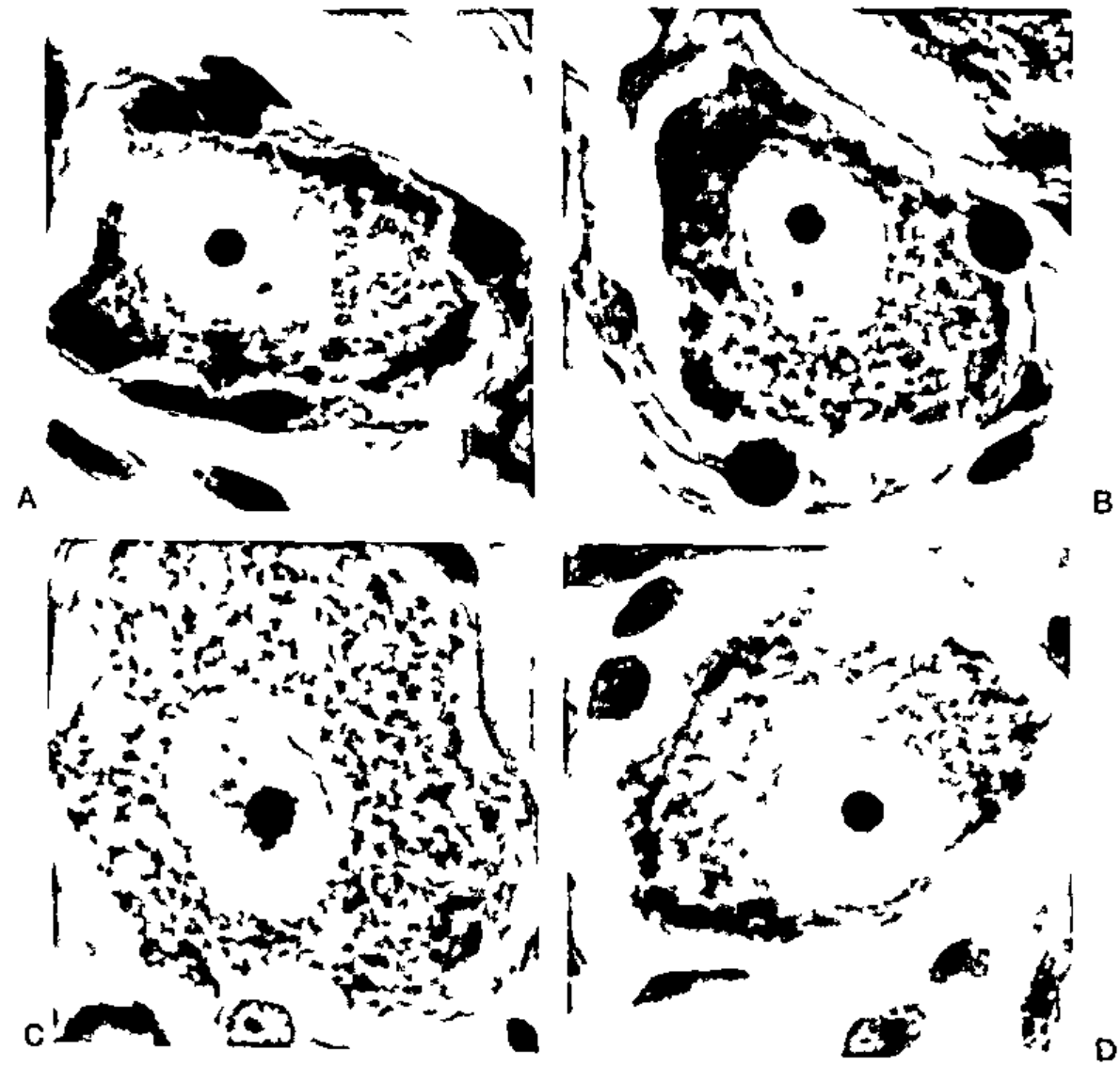


Fig 5 5A D Different locations of the sex chromatin in the neurons of the cat (*A*) Close to the inner surface of the nuclear envelope (*B*) Free in the nucleus (*C*) close to the nucleolus (*D*) no sex chromatin present (× 1600) (Courtesy of Professor M L Barr Reprinted by permission of Academic Press New York)

erochromatin has also been termed **satellite DNA** (Yunis and Yasmineh, 1970, 1971) It is thought that satellite DNA is composed of relatively short, repeated polynucleotide sequences (Walker and McClaren 1965, Britten and Kohne, 1968) This heterochromatin is also referred to as **redundant or repetitive.** It occurs commonly among eukaryotes It comprises some 10% of the genomes of higher organisms Both chromatins have the formation of interphase chromocenters and late DNA replication in common

The discussion of sex chromatin is mainly related to the facultative heterochromatin Sex chromatin is found in 20% to 96% of the nuclei of all females in humans and many mammalian species, but it is absent or rarely found in the nuclei of males of the same species Sex chromatin is generally located at the periphery of the interphase nucleus away from the main chromatin mass just inside and close to or flattened against the inner surface of the nuclear envelope (Fig 5 5) But it also can be located at other sites of the nucleus The size of this body is about 0 8x1 1 μm As mentioned, the first suggestion of the possible relationship between the sex chromatin and one of the two X chromosomes present in females was made in 1959 The basis for this assumption was the fact that whenever two Xs were found in the karyotype of an organism, a Barr body was detected in interphase nuclei A minimum of two X chromosomes is the prerequisite for the presence of a Barr body and if more than two X chromosomes are present, more than one Barr body can be expected in at least some of the interphase nuclei The ease with which sex chromatin now can be detected as in scrapings from the oral mucosa (Marberger et al , 1955, Moore and Barr, 1955) makes it a most valuable tool for sex diagnosis

Another method for sex diagnosis is the determination of the presence or absence of the so-called **drumsticks.** These were first discovered by Davidson and Smith (1954) in the circulating polymorphonuclear neutrophil leucocytes of human blood These are drumstick-like chromatin appendices that are attached by a fine chromatin thread to one lobe of the polymorph nucleus Drumsticks do not vary with

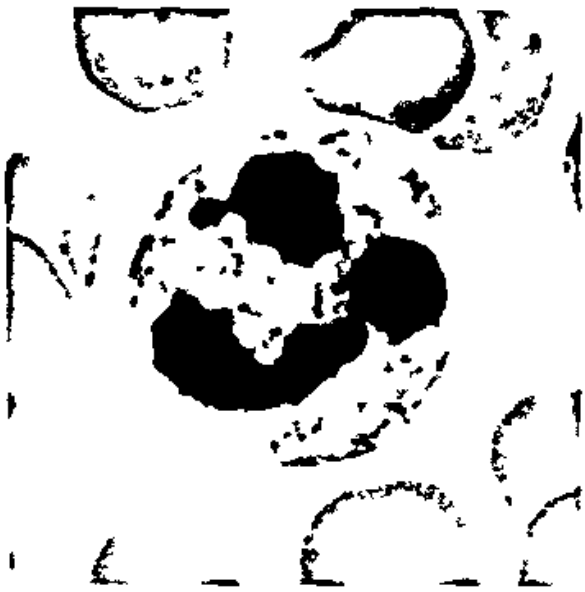


Fig 5 6 Chromatin appendixes called "drumsticks" of the neutrophil leucocytes of human female Drumsticks do not vary with age (× 2666) (Courtesy of Carolina Biological Supply Co Burlington N C)

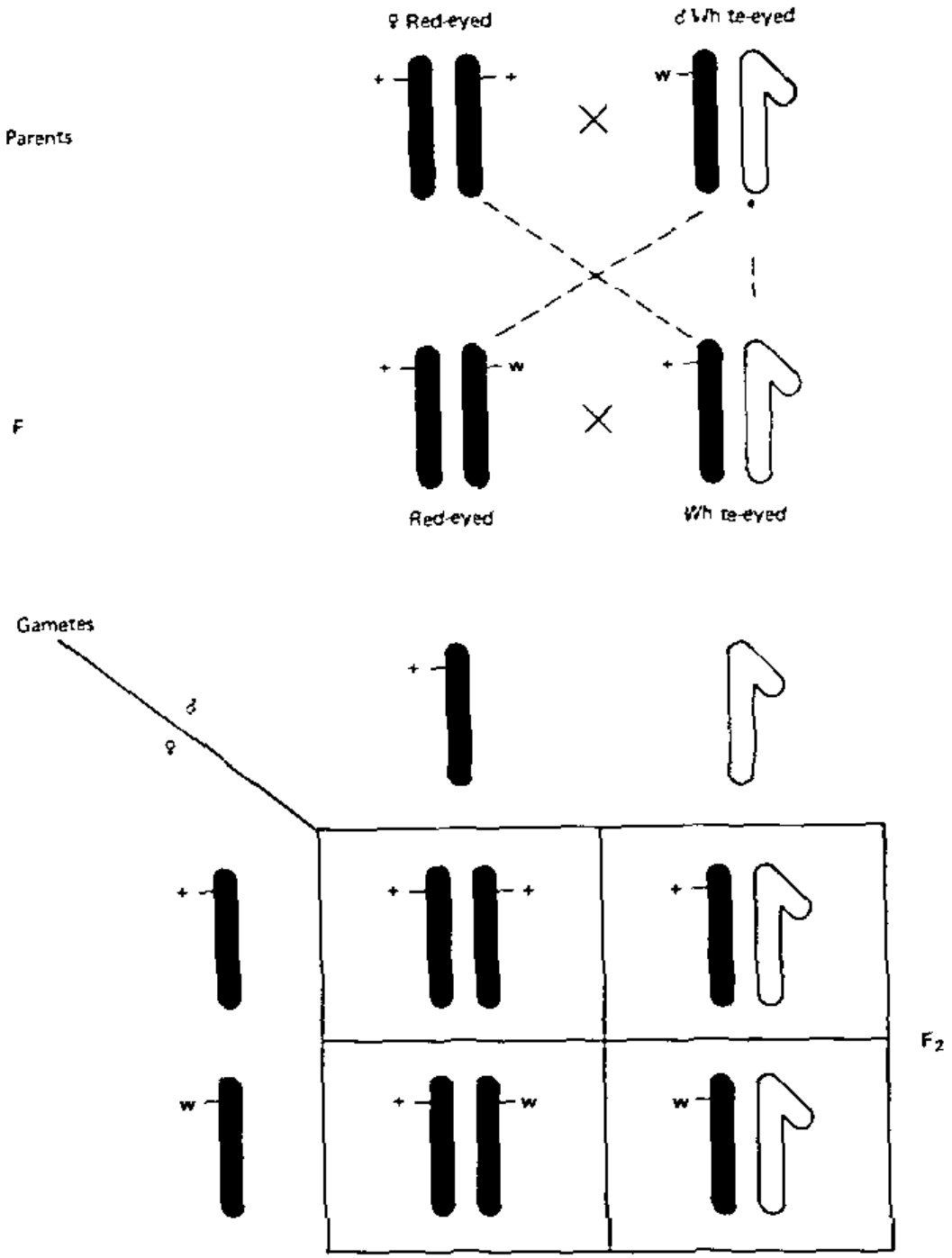


Fig 5 7 Diagram of a cross between a red-eyed female (+/+) and a white-eyed male (*w*/*y*) the resulting F_1 (+/*w* +/*y*) and the F_2 from a cross between the F_1 individuals This diagram demonstrates sex linkage (After Morgan 1910b)

age (Fig 5 6) Their size is 1 4 μm to 1 6 μm in diameter Drumsticks are found in about one out of 40 leucocytes of normal females and in less than one in 500 cells of normal males It has been hypothesized that drumsticks correspond to sex chromatin in that they represent the heteropycnotic region of the X chromosome It is, however, doubtful if such a conclusion can be made at this time

Sex chromatin and drumsticks are both valid structures for sex diagnosis although

Part IV
Movement of Chromosomes

Chapter 6
Chromosomes During Mitosis

In this part on the movement of chromosomes, the so-called **normal behavior** of the chromosomes during the processes of cell division and cell union will be discussed These two processes guarantee the continuation of species from one generation to the next

In cell division there exist two major phases, **karyokinesis** (or **mitosis**) and **cytokinesis** The term **mitosis** is usually preferred over **karyokinesis.** During mitosis the hereditary information that is contained in the chromosomes is passed on to the daughter nuclei During cytokinesis, which usually follows mitosis, the cytoplasm and its inclusions are divided finalizing cell reproduction Since the chromosomes, as the carriers of the hereditary units, are the major topic of discussion, only mitosis is treated in this chapter The principal stages of mitosis are **prophase, metakinesis, metaphase, anaphase,** and **telophase.** However, in order to understand the entire cell cycle, **interphase** is included in this discussion Figure 6 1 depicts the different mitotic stages in an animal cell

6 1 Interphase

In the interphase nucleus, the chromatin, the substance that contains the genetic material appears to be dispersed throughout the entire nucleoplasm The nucleolus and the chromocenters (Chapter 2) stand out by their dense staining properties In terms of the cell cycle, the interphase takes up a relatively long time interval The reason for this long period is that during interphase some very important functions of the cell cycle are taken care of, such as metabolism and synthesis The interphase period is generally subdivided into three phases—the G_1 period the S period (synthesis), and the G_2 period (Howard and Pelc, 1953) The length of these periods varies according to species and probably also according to different tissues of the same species In Fig 6 2, the relative duration of these interphases is indicated along with the estimated period of mitosis for cells such as cultured cells of man and hamster, root tip cells of the broad bean *(Vicia faba)* and spermatogonia and spermatocytes of grasshoppers The duration of the cell cycle in these organisms is 18 to 19 hours, while the S period lasts 6 to 8 hours During the S period, some chromosomes are early replicating and others are late Heterochromatic chromosomes such as the allocyclic X chromosome mentioned in Chapter 5 are usually

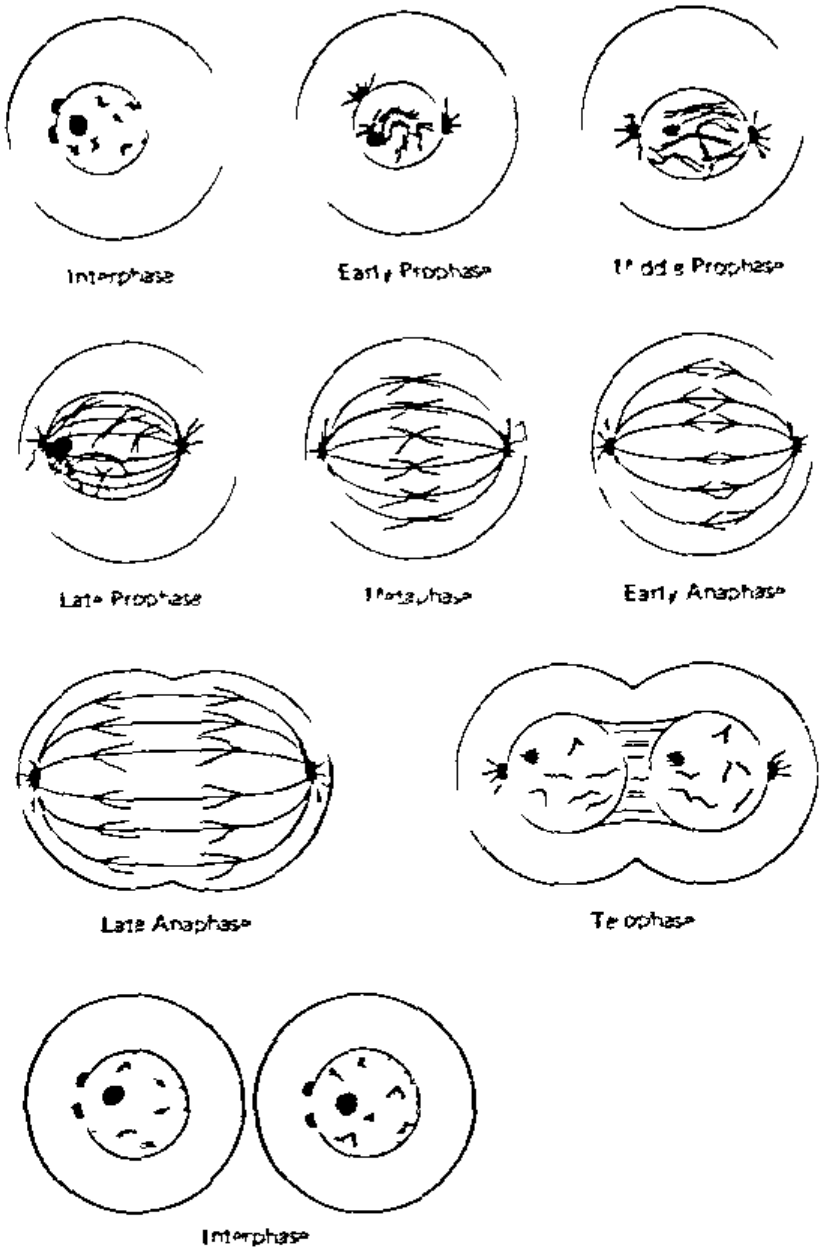


Fig 6 1 Mitotic stages in an animal cell

late replicating The S period is usually preceded by a presynthetic gap period called G_1 The physiological condition of the cell determines the length of the G_1 period Some cells such as inactively growing yeast may not have a G_1 period During the G_1 period the chromosomes are not reduplicated This period can be considered as a preparatory period for DNA synthesis The chromosomes are released from their condensed condition, which they had assumed during mitosis It has not been possible as yet to ascribe with certainty any specific biochemical events to either the G_1 period or the G_2 period (John and Lewis, 1969) Both RNA and protein synthesis are evident during the G periods as well as during the S period Near the end of the G_1 period, the beginning of the S period is triggered by a yet unknown event This is a period of active DNA synthesis during which the chromosomes replicate The S period can be traced by the use of labeled DNA

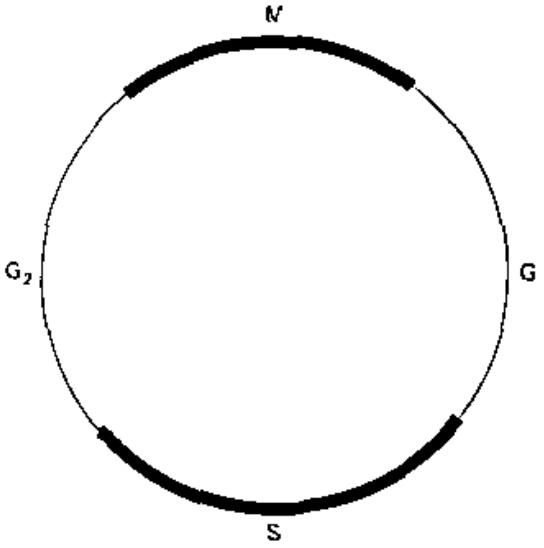


Fig 6 2 Life cycle of dividing cells such as those of humans hamster root tips of the broad bean and spermatogonia and spermatocytes of grasshoppers Phases indicated are mitotic division (M) synthesis (S) gap between M and S (G_1) and gap between S and M (G_2) (After Swanson et al 1967 Redrawn by permission of Prentice Hall Inc Englewood Cliffs N J)

precursors such as thymidine Several enzymes catalyze the process of DNA replication The most critical one is *DNA polymerase* This enzyme copies each strand of the DNA double helix into a complementary strand Since the DNA strand in a human chromosome has an estimated length of 30 000 μm and DNA replication occurs at a calculated speed of 0 5 μm per minute DNA replication would take much too long to account for a typical S period of 6 to 8 hours assuming replication proceeds sequentially from one end to the other But it was discovered that DNA replication occurs at many different places along the chromosome at once During synthesis short transient DNA fragments were observed that are called **Okazaki pieces** (Okazaki et al 1968 Huberman and Riggs 1968) which correspond to the earlier hypothesized **replicons** (Jacob and Brenner 1963) Such Okazaki pieces are autonomous DNA units with an average estimated length of about 1000 nucleotides or 30 μm that are eventually joined together by the action of DNA ligases forming the final product the complete DNA daughter strand The post synthetic G_1 period is a gap between synthesis and mitosis During this period the chromosomes are in a reduplicated state Irradiation experiments have verified this When irradiated during G the chromosomes yield chromosme aberrations and when irradiated during G_2 chromatid aberrations During the interphase directly preceding meiosis the G_2 period is either very short or completely missing Duration of the interphase periods G S and G_2 in relation to the mitotic period have been determined for many species A recent summary for higher plants was published by Van t Hof (1974)

6 2 Preparation for Mitosis

One of the preparatory phenomena of the cell for mitosis is cellular growth (Mazia 1961) A product of a mitotic division such as a late telophase daughter cell usually almost doubles its volume by the end of interphase before it divides again This applies particularly to cells in mitotically active tissues It demonstrates that mitosis is a cellular process and not entirely limited to the nucleus The preparation

Fig 6 3 Cross section of a centriole from a human lymphocyte at the interphase stage The 9 triplet fibers consist of 3 microtubules each (× 316 000) (From Sitte 1965)

for mitosis is going on continuously throughout the life of the cell Preparation for the next cell division already has started during the course of the previous division There are many preparations for a given division which all have to be completed before the mitotic apparatus becomes functional Bradbury et al (1974a 1974b) proposed that during the G period the initiation of mitotic cell division is controlled by the level of the growth associated enzyme F1 histone phosphokinase (**HKG**) but direct proof is not yet available Another preparatory process for mitosis is the replication and poleward movement of the **centrioles** of the **centrosome** (Boveri 1888)

6 2 1 The Centrosome

The centrosome which contains the centrioles is a region of clear cytoplasm adjacent to the outer side of the nuclear membrane of cells in many animals and in some lower plants (Boveri 1895) It was first described by Beneden in the 1870 s and by Boveri in 1888 in his famous *Cell Studies* mentioned in Chapter 1
Centrioles have been studied in detail with the electron microscope (Fig 6 3) They are shaped like a short hollow cylinder about 300 nm to 800 nm long and 160 nm to 250 nm in diameter The wall of the cylinder contains 9 triplet fibers that consist of 3 microtubules each The microtubules are about 15 nm to 20 nm in diameter The three microtubules of each triplet fiber are arranged in a line tilted about 30 to 40 to the tangent of the circumference of the centriole
During the G period of interphase usually two centrioles are observed They are generally replicated during the S period The ultrastructure of centriole replication was first described by Bernhard and DeHarven (1960) and Gall (1961)
The new centriole arises as smaller **procentriole** at an angle greater than 90 to the mother centriole forming an L shaped angle with the mother (Fig 6 4) The diameter of the procentriole is almost equal to that of the mother but the length is only about 70 nm when it first becomes visible under the electron microscope The procentriole gradually increases in size until it reaches the dimensions of the mother centriole In most organisms centriole replication is finished by the end of interphase (G_2 period) At that time two pairs of centrioles or two **centriole duplexes** are visible at one side of the nuclear envelope (Fig 6 5)
At the beginning of prophase one centriole duplex starts to move away from the other one and migrates around the periphery of the nucleus The other centriole

Fig 6 4 Longitudinal sections of centrioles from the alga *Nitella* The new centriole arises at an angle greater than 90° to the mother centriole forming an L-shaped angle with the mother × 52 200 (Micrograph from Turner 1968 Reprinted by permission of the Rockefeller University Press New York)

duplex remains in its previous position During this time small spindle fragments appear between the separating centriole pairs

At the beginning of metaphase the migrating centriole pair has obtained a position opposite to its two partners A complete spindle fiber system formed by the asters now stretches from centriole pair to centriole pair During anaphase the centriole pairs seem to be pushed farther apart by the continuous spindle fibers

There may be some exceptions to this general centriole behavior described above

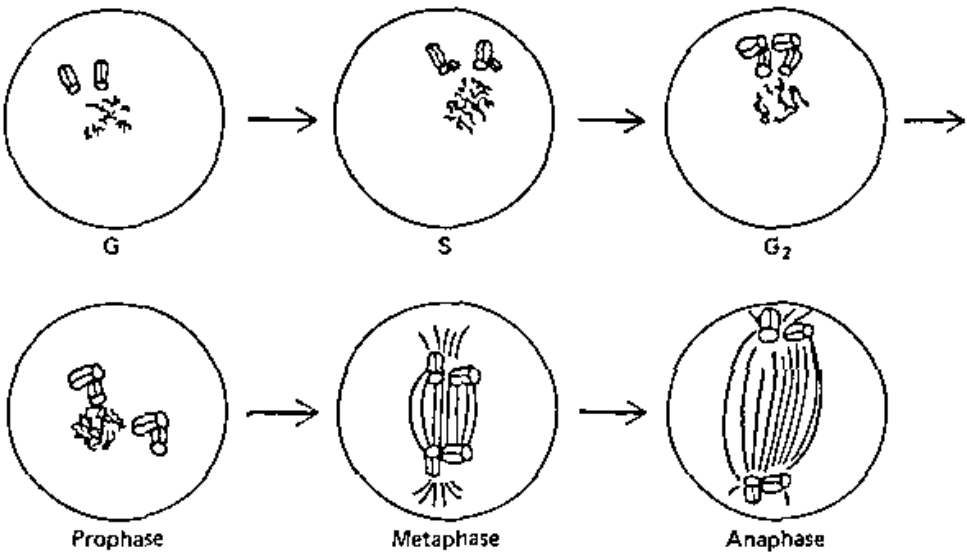


Fig 6 5 The centriole cycle during interphase and cell division (Modified after *Elements of Cytology* Second edition by Norman S Cohn 1969 by Harcourt Brace Javanovich Inc Redrawn by permission of the publisher)

The centriole duplex separation may be delayed beyond the beginning of prophase (Fig 6 5) Both centriole duplexes may migrate around the cell as a unit with little change in their center to center spacing prior to separation Duplex separation may occur at any point within the mid prophase prometaphase period (Rattner and Berns 1976)

The function of centrioles seems to be connected with the formation of the spindle fiber mechanism and the detecting of chromosome migration during mitosis The centriole is the place where the tubulin (Borisy and Taylor 1967) is assembled and serves as the center of the spindle microtubules (spindle fiber) organization Tubulin is the subunit protein of the microtubules

6 3 Prophase

Prophase is the beginning stage of mitosis During this stage the chromosomes become visible as thin threads This is accomplished by progressive coiling and folding Each prophase chromosome now consists of two adjacent chromosome threads called **chromatids**, which are the result of chromosome reduplication dur ing the S period of interphase The coiling and folding transform the largely extended metabolic chromosomes into a shape suitable for transport A diagram matic representation of the coiling cycle is shown in Fig 6 6 During early prophase the two chromatids are twisted about each other in **relational coils** (No 2 Fig 6 6) The coils interlock in such a manner that the chromatids cannot be separated without unwinding the coil This kind of twisting is also called **plectonemic coiling** (Fig 6 7A) Such chromatid association is different from the one occurring in meiotic prophase, which is called **paranemic coiling** (Fig 6 7B) where the chromatids are easily separated laterally (Sparrow et al , 1941) As prophase proceeds and the chromosomes become shorter, the relational coiling disappears, the chromatids disengage themselves and lie side by side (No 4, of Fig 6 6) Later, during the coiling cycle (metaphase and anaphase), two levels of coiling can be seen (No 6, Fig 6 6) The large coils are called **somatic coils**, the small ones that are imposed upon the large ones are called **minor coils**. As seen in the diagram, the initially small somatic coils decrease in number with progressing prophase and at the same time increase in diameter This causes an apparent thickening of the chromosomes that is often referred to as contraction

During prophase, the nucleolus of most species breaks down and disappears Electron microscopic studies have revealed that the component parts of the nucleolus disperse throughout the nucleus during this stage In some lower forms of life the nucleolus persists through metaphase and anaphase and divides into two halves that are distributed to the daughter cells

At the end of prophase, the nuclear envelope breaks down into fragments This allows the chromosomes to spread over the greater part of the cell and gives them a better chance to separate as chromatids during poleward movement Electron microscopic investigation seems to prove that pieces of the nuclear envelope dis perse into the cytoplasm and become part of the **endoplasmic reticulum** It is pos sible that the nuclear envelope originates from the endoplasmic reticulum (Bern

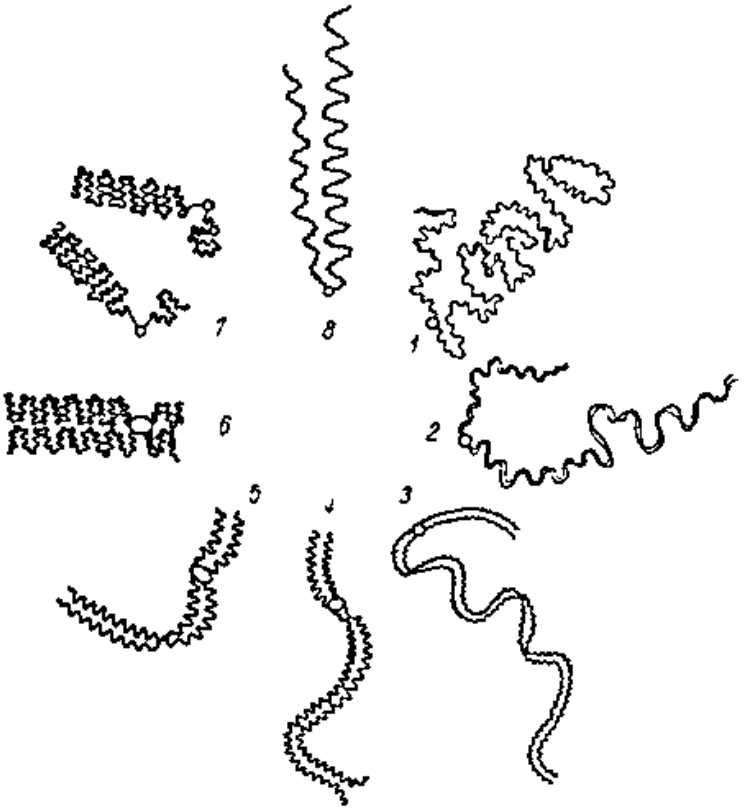


Fig 6 6 Diagrammatic representation of the mitotic coiling cycle of a chromosome Centromeres are shown as circles—(1) Interphase (2, 3, and 4) Prophase (5) Prometaphase (6) Metaphase metaphase chromatids show major and minor coils (7) Anaphase (8) Telophase (Modified after De Robertis et al , 1965)

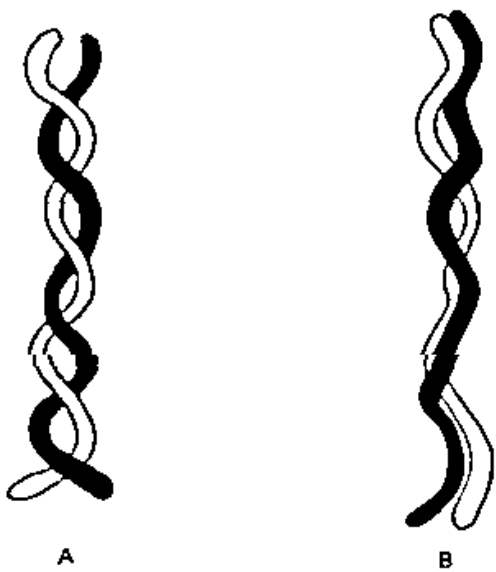


Fig 6 7A and B Diagram of two possible types of coiling between chromosomal subunits (*A*) Plectonemic coiling (*B*) Paranemic coiling

hard 1959 Whaley et al 1960 Porter 1961) Protozoa and fungi comprise an exception in that the nuclear envelope remains intact throughout the entire mitotic division
Right after the disappearance of the nuclear envelope the spindle fiber apparatus appears

6 4 Metakinesis

The term **metakinesis** was first used by Wassermann in 1926 and was brought into popular use by Mazia (1961) Many textbooks do not distinguish between metakinesis and metaphase but include the discussion of both of these stages under metaphase But many of the important features of metakinesis are omitted from the discussion if it is not considered separately Metakinesis is often also referred to as **prometaphase** (Lawrence 1931)
Darlington (1937) divided the movement of the chromosomes during the metaki netic stage into three substages

1 chromosome congression
2 centromere orientation
3 chromosome d stribution

6 4 1 Chromosome Congression

During **chromosome congression** the chromosomes move to the equatorial plate half way between the two poles of the spindle where the centriole pairs are located The chromosomes reach a position of equilibrium at the equatorial plate In gen eral this movement is coordinated except in instances where small chromosomes perform their movement out of step with the large ones in the same complement Detailed cinematographic studies by Bajer and Mole Bajer (1954 1956) show that individual chromosomes may move toward the pole at first then make a turn and finally arrive at the equator The movement toward the equator may be very abrupt The chromosomes are now freely floating in the cytoplasm unrestricted by the nuclear envelope In the grasshopper neuroblast this metakinetic movement lasts only four minutes out of a total duration of mitosis of three hours (Carlson and Hollaender 1948) It is possible that the spindle fibers are required for the movement of the chromosome toward the equatorial plate since they are not able to migrate when the spindle has been destroyed with the spindle fiber poison col chicine (O Mara 1939 Eigsti 1942 Berger and Witkus 1943 Allen et al 1950 Hadder and Wilson 1958 Malawista et al 1968)

6 4 2 Centromere Orientation

This metakinetic movement was described in detail by Darlington (1936) It deals with the orientation of the **kinetic sites** of the chromosomes toward opposite poles through movements that lead toward their orderly arrangement in the equator (coorientation) Each metaphase chromosome consists of two chromatids and each

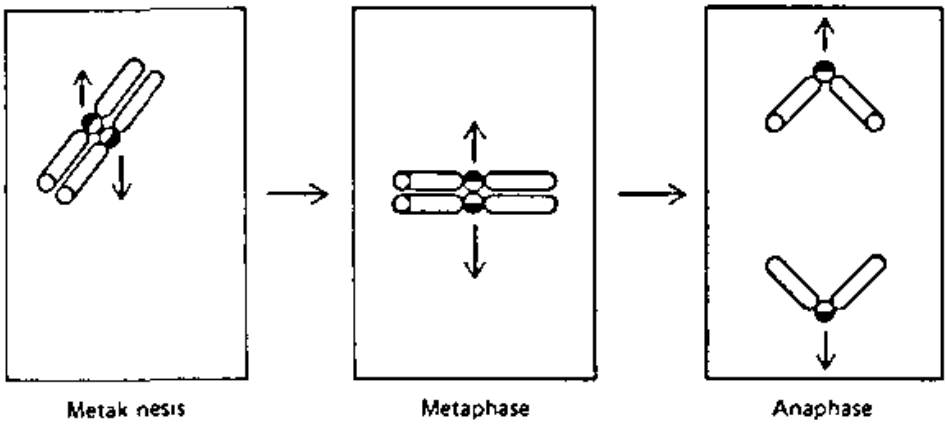


Fig 6 8 Auto-orientation of mitotic chromosomes (From Rieger and Michaelis 1958)

of these chromatids has a kinetic site and an **akinetic site** (Fig 6 8) The kinetic sites are oriented toward the poles through forces that originate from the poles and that very well could be the spindle fibers While the chromosomes previously were located in the cell at random, centromere orientation places them into a stable equilibrium at the equator According to Darlington, the same forces that accom plish centromere orientation are responsible for moving the chromosomes toward the poles at anaphase (Fig 6 8)

6 4 3 Chromosome Distribution

The third process of metakinetic movement is **chromosome distribution.** The centromeres come to be oriented in such a way that their corresponding chromosomes are more or less evenly distributed on the equatorial plate Darlington thinks that this even distribution of the chromosomes is caused by some kind of body repulsion The chromosomes are not always distributed at random on the equatorial plate, but they may be subject to specific arrangement This was already known by such prominent cytologists as Wilson (1925) and Schrader (1953) The metaphase chromosomes of many insects are arranged in such a way that the larger chromosomes lie on the periphery of the equatorial plate while the smaller chromosomes lie in the middle On the other hand, there is also the reverse tendency such as observed in human cells were the largest chromosomes, numbers 1 and 2, were found near the middle while the smaller chromosomes, Y and numbers 13, 17, 18, and 21, lay near the periphery (Miller et al , 1963)

6.5 Metaphase

At metaphase the chromosomes are at their highest level of coiling and therefore appear to be shorter and thicker than in any other stage This makes them ideal for cytotaxonomic studies because they are most sharply defined during this stage (Chapter 2, Section 2 1) There is no longer much relational coiling present, and, consequently, the chromatids are no longer twisted about each other but lie side

Fig 6 9A and B C pairs in mitotic metaphase of *Allium cepa* (2n = 16) Four hours of treatment with 0 2% colchicine solution (*A*) Uncoiling of colchicine treated chromosomes has reduced the number of turns in each chromosome arm producing figure 8 and forceps types (*B*) Cross type c pairs are only connected at the centromere (× 1258) (Schulz Schaeffer unpublished)

by side (No 6 Fig 6 6) Proof of this conclusion is the ease of separation of the chromosome arms as a result of colchicine treatment which leaves the chromosomes only attached at the undivided centromeres Such colchicine influenced chromatid associations in metaphase are called c-pairs (Fig 6 9) and have a cross shaped appearance (Levan 1938)

Metaphase is much shorter than prophase but on the average somewhat longer than anaphase Table 6 1 gives a comparison of the length of mitotic stages of different tissues of a number of animal and plant species

The end of metaphase is signaled by an almost simultaneous splitting of the cen*tromeres and separation of all sister chromatids at the centromeres Brown (1972)* writes that somehow all the chromosomes *know* when to separate and start anaphase and that they all do this at the same time even if most of them have to wait for one laggard to arrive late on the metaphase plate The controlling mechanism for this separation remains to be discovered

6 6 Anaphase

Anaphase is a stage of active and rapid movement and is the shortest of all mitotic stages (Table 6 1) During this stage the spindle elongates and the centriole duplexes—if present—move closer to the cell periphery (Fig 6 5) As the centro-

Chapter 7
Chromosomes During Meiosis

As mentioned in Chapter 1, the essential facts of meiosis and fertilization in animals and plants were demonstrated by Beneden (1883), Strasburger (1884), Boveri (1890) and Oscar Hertwig (1890) These investigators found that the most important result of fertilization was the fusion of gametes of maternal and paternal origin Since the nuclei of a particular species maintain their constant chromosome number (2n) from generation to generation, they concluded that a mechanism had to operate that would compensate for the increase of chromosome number during fertilization This mechanism was found to be a reduction of chromosomes before fertilization, as it occurs in **meiosis** of higher plants and animals Other essential characteristics of meiosis are the pairing of chromosomes, which makes reduction possible, and crossing over as discussed in Chapter 4, which provides for recombination

Meiosis includes two nuclear divisions that generally succeed each other rapidly and during which the chromosomes divide only once In spite of modified types of meiosis having been observed the details of the basic process are very similar for humans and for the majority of higher animals and plants These two divisions have been called different names according to the different functions carried out during these divisions by the chromosomes Names like **heterotypic** vs **homeotypic** as well as **reductional** vs **equational** division are some more familiar terms The first of the two divisions has been called *heterotypic* because it is the more unusual one while the second was called *homeotypic* because it is more similar to a normal mitotic division The terms "reductional" for the first and "equational" for the second meiotic division are misnomers and would be correct if crossing over would not occur In the presence of crossing over, however, portions of the chromosomes divide reductionally during the first meiotic division (**prereductional separation**), while other portions divide reductionally during the second meiotic division (**postreductional separation**). During reductional separation, homologous segments of non sister chromatids disassociate, while during equational separation, homologous segments of sister chromatids separate Logically, there is also **preequational** and **postequational separation.** These phenomena are illustrated in Fig 7 1 During the first meiotic division (MI) of this illustration, a predominantly paternal chromosome (black) moves away from a predominantly maternal chromosome (white) in a reductional fashion, while portions that have been exchanged by crossing over separate from one another in an equational fashion (black from black and white

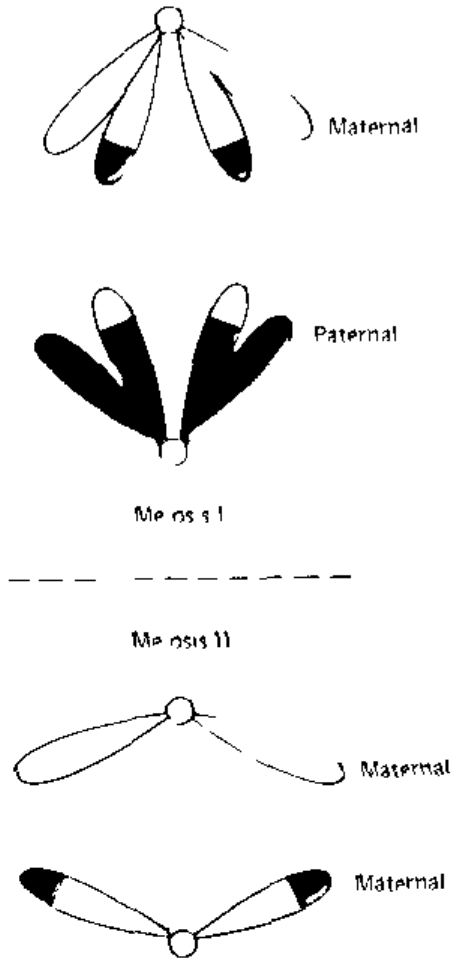


Fig 7 1 Prereduction and postreduction in meiosis Chromosome separation is predominantly reductional in meiosis I of this illustration (white segments separating from black ones) Only small segments separate equationally (white from white and black from black) Chromatid separation in meiosis II is predominantly equational in this illustration

from white) During the second division a predominantly maternal chromatid (white) moves away from a predominantly maternal chromatid (white) in an equational fashion while small portions (black vs white) divide reductionally In reality, none of the two meiotic divisions are truly reductional or equational The real condition depends on the crossover situation of a given meiosis

The most commonly used nomenclature for the two divisions are the terms **meiosis I** (MI) and **meiosis II** (MII) or first and second meiotic divisions In order to understand meiosis, some other terminology should be introduced here The prophase of the first meiotic division usually is of long duration since **homologous chromosomes** (homologues) are pairing during this period in **synapsis**. Since each partner of such a pair of sister chromatids (reduplication occurred in the preceding synthesis period as described for mitosis), there are now four chromatids present immediately after synapsis Such a group of four chromatids is generally referred to as a **tetrad chromosome** (Nemec, 1910) (Fig 7 2) A tetrad chromosome is also called a **bivalent** referring to the two homologous chromosomes belonging to it During anaphase I the tetrad chromosome separates into two **dyad chromosomes** (Nemec 1910), each of which consists of two chromatids Such a dyad chromosome can also be called a **univalent.** During anaphase II the dyad chromosomes divide into **monad chromosomes** or unit chromatids This completes the entire cycle of two meiotic cell divisions (Fig 7 2)

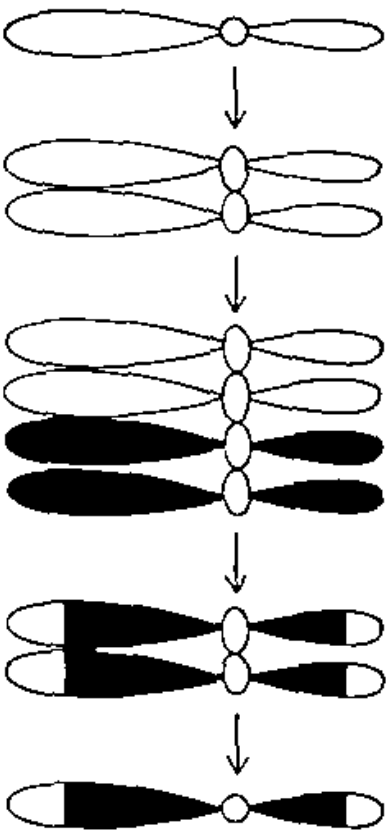


Fig 7 2 The formation of dyad chromosomes tetrad chromosomes and monad chromosomes during a regular meiotic cycle

Other relationships during meiosis and mitosis can be expressed in terms of total DNA present during these different stages For this purpose the **C-value** has been employed If C represents the amount of DNA in a haploid gamete before fertil ization, then a gamete will have an amount of 1C and a zygote of 2C (Fig 7 3) During the S period of premeiotic mitosis the DNA content in the cell will rise to 4C Mitotic anaphase will bring it back down to 2C During the S period immediately preceding meiosis it will rise back to 4C Anaphase I will reduce the DNA content to 2C and anaphase II to the original 1C value

Meiosis and gametogenesis (Chapter 8) occur only in special tissues of organisms that during development are differentiated and are set aside as gamete forming tissues Weismann in 1883 and 1885 in his germ plasm theory (see Chapter 1) called this special tissue the **germ line**.

Meiosis like mitosis has been divided into stages and substages They are called

- Premeiotic Interphase
- Prophase I
  - Leptotene
  - Zygotene
  - Pachytene
  - Diplotene
  - Diakinesis
- Anaphase I
- Telophase I
- Interkinesis
- Prophase II
- Metaphase II
- Anaphase II
- Telophase II

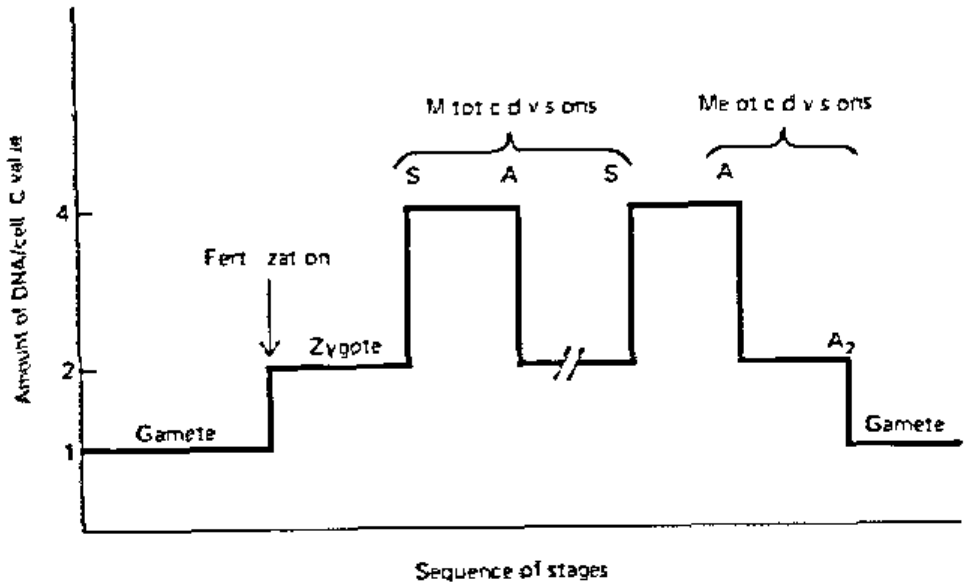


Fig 7.3 Increase and decrease of the C value during mitotic and meiotic cycles of animals and plants S synthesis A anaphase (From Swanson et al 1967 Redrawn by permission of Prentice Hall Inc Englewood Cliffs N.J.)

A schematic drawing of a homologous pair of chromosomes passing through these stages is represented in Fig 7.4

7.1 Premeiotic Interphase

Premeiotic interphase is very similar to premitotic interphase in that during the S period the chromosomes are reduplicated Compared with the mitotic S period the meiotic S period is longer

In cells of anthers of lily *(Lilium longiflorum)* and tulip *(Tulipa gesneriana)* a unique histone was found that was absent or nearly so from the somatic tissues of these plants (Sheridan and Stern, 1967) This histone was therefore called **meiotic histone.** It is synthesized during the S period of premeiotic interphase and persists through meiosis, microsporogenesis, and pollen maturation The possible function of a meiotic histone is not known But histones are thought to be involved in some way or another in the regulation of genetic activity The discoveries of Huang and Bonner (1962) have been discussed in this connection in Chapter 1 We know that the entire process of cell division in general and of meiosis specifically is under rigorous control of certain genes or groups of genes It is not hard to follow that a specific meiotic histone would have a certain function in the regulation of the rather specific phenomena of meiosis Very few analyses of meiotic histones have been reported so far, and future research in this area seems promising

The duration of the different stages of meiosis varies from species to species A summary of data from some higher plants was recently published by Van't Hof (1974) and is shown in Table 7.1 If these data are compared with Table 6.1, it can be seen that meiosis in general, and its first division in particular, lasts considerably longer than mitosis

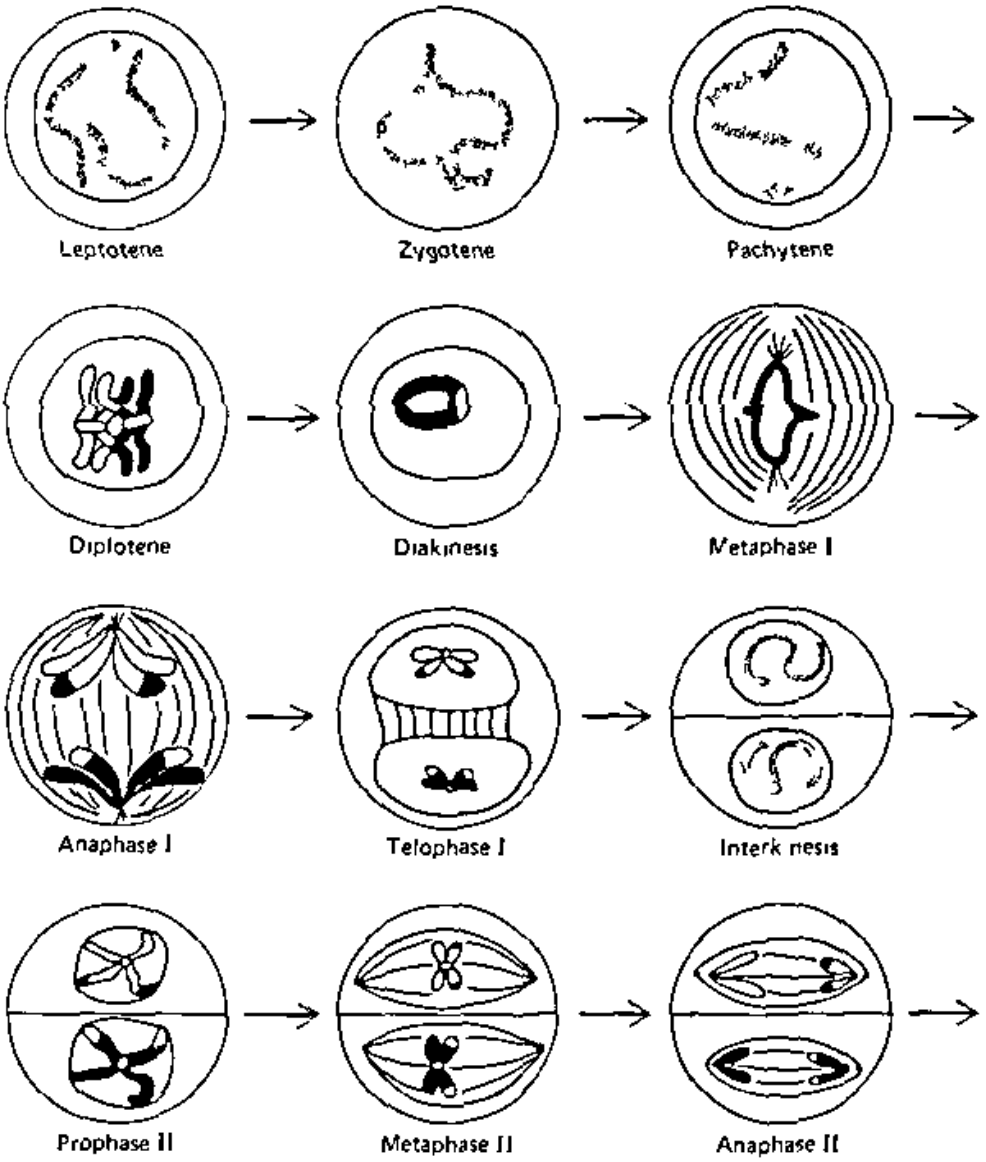


Fig 7 4 Schematic representation of a pair of homologous chromosomes passing through the stages of meiosis

7 2 Prophase I

An important feature of Prophase I is the great increase in volume of the nucleus This increase is greater than that during mitosis and is partly due to an increase in hydration which is several times greater than in mitosis According to Beasley (1938) the volume of the meiotic prophase nuclei in plants and animals is 3 to 4 times that of mitotic prophase nuclei Prophase I of meiosis is also of extremely long duration if it is compared with mitotic prophase (Table 6 1 and Table 7 1) The chromosomes have to perform specific functions during this period that they do not have to carry out during mitotic prophase Such functions are chromosome pairing chromatid exchange, repulsion, and terminalization According to these functions, prophase I is divided into 5 substages leptotene, zygotene, pachytene, diplotene, and diakinesis These stages are not rigid entities but rather arbitrary

Table 7 1 The duration of stages of meiosis of 14 plants[a]

Species	Duration of meiotic stages, hr													
	Leptotene	Zygotene		Pachytene	Diplotene	Diakinesis	Metaphase I	Anaphase I	Telophase I	Dyads	Metaphase II	Anaphase II	Telophase II	Total meiotic duration	Ploidy level
Antirrhinum majus	6 0	3 0		3 0						9 0				2 4 0	
Allium cepa			7 2 0							2 4 0				9 6 0	
Hordeum vulgare	1 2 0	9 0		5 8	2 2	0 6	1 6	0 5	0 5	2 0	1 2	0 5	0 5	3 9 4	2x
Lilium candidum	4 0 0	4 0 0		4 0 0						2 4 0				1 6 8 0	2x
Lilium longiflorum	4 0 0	3 6 0		4 0 0						4 8 0				1 9 2 0	2x
Rhoeo discolor			2 4 0							2 4 0				4 8 0	2x
Secale cereale	2 0 0	1 1 4		8 0	1 0	0 6	2 0	1 0	1 0	2 5	1 7	1 0	1 0	5 1 2	2x
Secale cereale	1 3 0	9 0		6 4	1 0	0 6	1 8	0 7	0 7	2 0	1 4	0 7	0 7	3 5 0	4x
Tradescantia paludosa	4 8 0	2 4 0		2 4 0						1 3 0				1 2 6 0	2x
Tradescantia reflexa			1 1 1 0							1 1 0				1 4 4 0	4x
Trillium erectum	7 0 0	7 0 0		5 0 0						6 4 0				2 7 4 0	2x
Triticum aestivum	1 0 4	3 4		2 2	0 6	0 4	1 6	0 5	0 5	2 0	1 4	0 5	0 5	2 4 0	6x
Triticale	7 5	3 0		2 3	1 0	0 5	1 5	0 5	0 5	1 5	1 3	0 5	0 5	2 0 8	8x
Tulbaghia violacea			1 0 2 0							2 8 0				1 3 0 0	2x

[a] Data from Bennett and Smith (1972) Bennett (1971 1972)

divisions of a continuum They serve mainly to help the student to better understand the functions

7 2 1 Leptotene

Leptotene does not differ very much from early prophase in mitosis with the exception that meiotic prophase cells are larger than mitotic ones Leptotene chromosomes are longer and thinner than those in early mitotic prophase Also during leptotene bead like structures called chromomeres (Section 2 2 2) are appearing along the entire length of the chromosomes Early observers such as Belling (1928) considered these structures to be the visible manifestations of the genes but other studies seem to substantiate that they are merely regions of the chromatin threads (chromonemata) that are more tightly coiled than the interchromomeric regions (Ris 1945) In Crick s (1971) chromosome model (see Fig 3 6) he interprets the gene regions (coding DNA) to be located in the interchromomeric regions (interbands) Other studies such as the very thorough investigation of the *zeste-white (zw)* chromosome region of the *Drosophila* X chromosome (Judd et al 1972) seem to indicated that there is a correspondence between the presence of an amplified chromomere (polytene band) and the presence of a gene In other cases several genes were located in a region of a polytene chromosome where only one band is present

In some instances particularly in animals a certain type of polarization has been observed in this stage in which the ends of the chromosomes seem to be attached to the nuclear envelope (Moens 1969b) at the site where the centrosome is located in animal cells This has been referred to as the **bouquet stage** (Eisen 1900) which can be observed in both leptotene and pachytene A barley cell in pachytene suggesting such arrangement is shown in Fig 7 5 It has been speculated that bouquet formation may aid in the union of homologous chromosomes during synapsis in the next stage zygotene A similar phenomenon has been described in plants where the chromosomes are densely clumped to one side leaving the rest of the nucleus clear Such clumping into a more or less dense knot has been referred to as **synizesis** (McClung 1905) or **synizetic knot** (Fig 7 6) It has been claimed by some that synizesis may be due to a fixation artifact An increase of size of the nucleolus during leptotene has been related to RNA and protein synthesis

7 2 2 Zygotene

As in mitosis the chromosomes during meiosis gradually become shorter in length and wider in diameter as a result of progressive coiling Coiling mechanisms were described in the last chapter Swanson (1957) states that in meiosis the coiling picture is comparable to mitosis but is more complicated and that this is due to the occurrence of synapsis and chiasma formation A greater degree of contraction is attained by the chromosomes in meiosis This greater contraction is partly accomplished by the **major coils** in metaphase I and anaphase I that are larger in diameter but fewer in number than the somatic coils (Chapter 6) in mitosis

Zygotene is primarily the stage of pairing of homologues This pairing is envisioned

Fig 7.5 A barley cell in pachytene suggesting bouquet arrangement of the chromosomes (Courtesy of Mrs Christine E Fastnaught McGriff, Department of Plant and Soil Science, Montana State University)

as being in a zipper-like fashion starting at any or even at several "contact points" along the chromosomes and proceeding until all homologous segments are in a pairing equilibrium Pairing is not always completely finished Exceptions are also those chromosomes that have nonhomologous sections such as the sex chromosomes (see Fig 5 1) But in general, meiotic pairing of homologous chromosomes is remarkably precise and specific and gene by gene Exceptions have been observed and summarized (Riley and Law, 1965)

The phenomenon of chromosome pairing is called **synapsis** and was apparently first observed by Moore in 1895 Synapsis is a prerequisite for an orderly separation of homologous chromosomes during first meiotic anaphase Electron microscope studies have revealed some remarkable insight into synapsis in recent years

Fig 7 6 Maize cell in leptotene Chromosomes are clumped into a dense synizetic knot (× 632) (Schulz Schaeffer, unpublished)

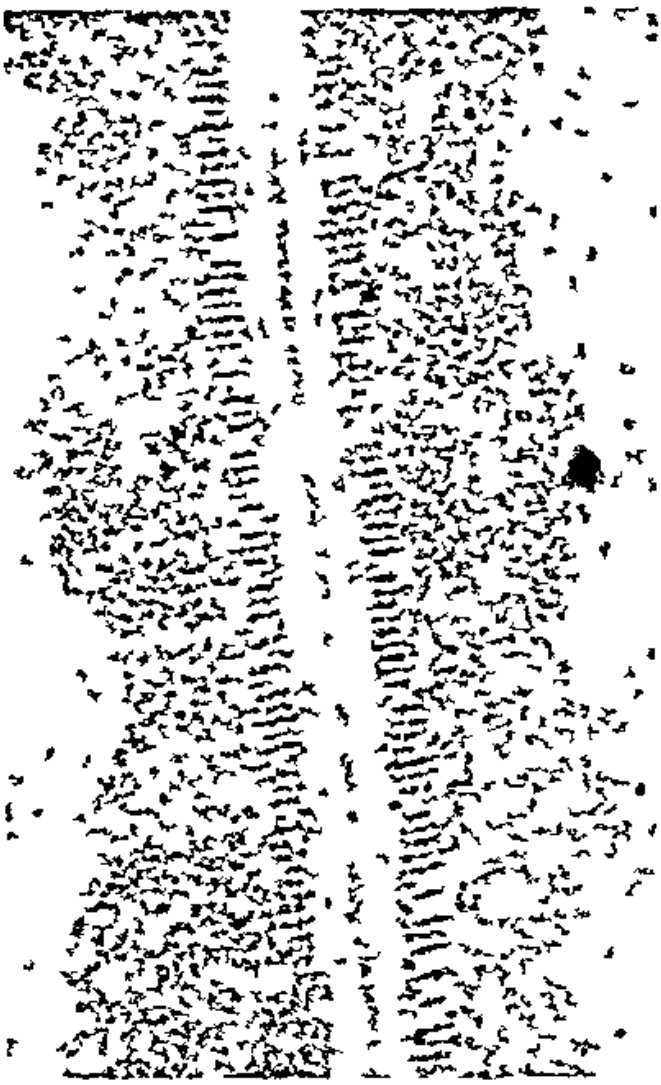


Fig 7 7 Electronm crograph of the ultrastructure of the synaptonemal complex of the ascomycete *Neott ella* (From Westergaard and W ettste n 1970 Reprinted by perm s s on of Carlsberg Laboratory Copenhagen)

Moses in 1956 first discovered a tripartate ribbon at the site of synapsis in cray fish called the **synaptonemal complex**

7 2 2 1 The Synaptonemal Complex (SC) Th s complex is one of the few con figurat ons in which the 10 nm fibers of the chromosomes are arranged into a superstructure that can be viewed under the electron m croscope It has now been established that this complex occurs in all animals and plant nuclei undergoing synapsis It is composed of three parallel electron dense elements that are sepa rated by less dense areas (Fig 7 7) The two **lateral elements** seem to be composed of fibers that are sl ghtly wider than 10 nm (**synaptomeres**) They vary in structure between different stages of meiotic prophase I w thin a species The **central ele ment** is a ladder like configurat on in the center of the SC It is more pronounced in some species than in others The **transverse elements** are electron dense fila ments that interconnect the central element with the lateral elements The lateral

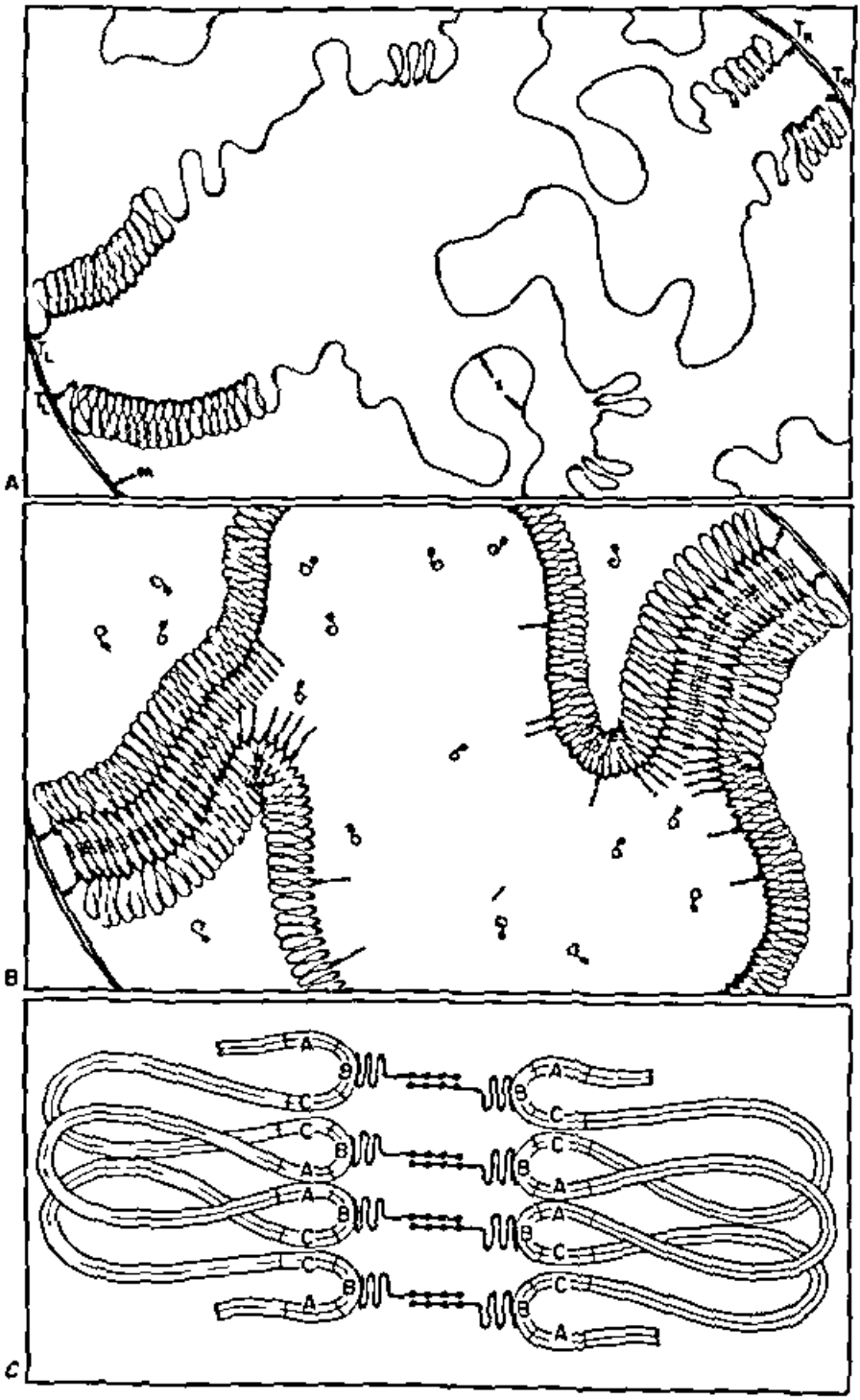


Fig 7 8 Illustration of the synaptomere zygosome hypothesis of synaptonemal complex formation (From King, 1970 Redrawn by permission of Academic Press, New York)

The exact nature of events that lead to synapsis is still not clear and is vigorously debated in the literature A strong group of investigators believes that homologous chromosomes are prepared for synaptic pairing by the attachment of their telomeres to so-called "attachment sites" on the nuclear envelope as mentioned in the above hypothesis (Wettstein and Sotelo, 1967, Woollam et al , 1967, Moens,

1973) Comings (1968) believes that these attachments are already evident in the interphase nucleus and that some of the attachment sites may correspond to the points of initiation of DNA synthesis in each replicon This viewpoint is called by Maguire (1977) the **nuclear envelope homologue attachement site model.** As an alternative to such a hypothesis Maguire proposes an **elastic connector model,** according to which homologous chromosome pairing may be accomplished by chance meeting of homologous chromosome segments followed by the establishment of elastic connectors at congression in premeiotic mitosis (Maguire, 1974) Similar connectors have been hypothesized by Holliday (1968) and Bennet et al (1974) Homologous chromosome segments may be connected in such a fashion in the stages intervening between premeiotic metaphase and zygotene **Premeiotic mitotic pairing** has been observed by quite a few investigators (Comings, 1968 Grell 1969 Dover and Riley 1973)
A special protein has been identified that may be involved in meiotic and mitotic chromosome pairing An increase of this protein coincides with the leptotene-to pachytene-period of meiosis Hotta and Stern (1971) called this substance **colchicine binding protein.**

7 2 3 Pachytene

Pachytene seems to be a stable stage in that the pairing of homologues is completed The homologues are closely appressed and form bivalents In pachytene the chromosomes are shorter than during early prophase and in well flattened cells can be distinguished as separate entities This made possible pachytene analysis as described earlier (Chapter 2) Figure 7 9 shows an exceptionally well spread pachytene cell of the grass *Bromus secalinus* (2n = 28) In Fig 7 10 a pachytene cell of a *Triticum durum* x *Agropyron intermedium* backcross derivative plant is shown This is the more typical situation in pachytene where individual chromosomes are hard to discern The arrow shows the paired nature of the bivalent threads At the middle of a pachytene, a longitudinal cleavage becomes apparent in each homologue This demonstrates that each pachytene bivalent consists of 4 chromatids forming a tetrade chromosome (Fig 7 2) The nucleoli are particularly evident during pachytene In many species they have already all united into

Fig 7 9 Pachytene cell of *Bromus secalinus* (2n = 28) (× 2111) (From Schulz-Schaeffer, 1956 Reprinted by permission of Verlag Paul Parey, Berlin)

Fig 7 10 Cell of *Triticum durum* × *Agropyron intermedium* back cross derivative (Arrow Paired nature of bivalent thread) (× 1338) (Schulz Schaeffer, unpublished)

one big nucleolus by pachytene that is attached to the nucleolus organizer chromosomes There seems to be evidence that DNA synthesis can extend into meiotic prophase and that it overlaps with synapsis and extends beyond it (Hotta et al, 1966) Such late DNA replication apparently has not been observed during mitosis and the question arises if such replication may be related to a chromosome break repair mechanism that may be connected with crossing over (see polaron hybrid DNA model Chapter 4) The major function of the chromosomes during late zygotene and pachytene is the phenomenon of crossing over, which has already been discussed (Chapter 4) This function has been closely related to the structural discovery of the synaptonemal complex (King, 1970 Wolfe, 1972) Wolfe concluded that the concept of the synaptonemal complex can be conveniently fitted into the phenomena of initial chromosome pairing close pairing recombination, and chiasma formation and can even be related to the features of both classical and intragenic recombination That the complex is involved in at least some of these processes seems certain Whether all of the listed mechanisms take place with the direct intervention of the complex remains to be seen (Wolfe, 1972)

7 2 4 Diplotene

During diplotene the chromosomes further contract and thicken (Fig 7 11) This is also the stage where the **chiasmata** (Chapter 4) become apparent as visible evidence of crossing over Figure 7 12 shows a diplotene cell of a *Triticum* x *Agropyron* derivative with evidence of chiasmata The synaptic attraction of the chromosomes suddenly comes to an end the homologues move apart in **repulsion** and are only held together at exchange points that are the result of crossing over Only two of the four chromatids are involved in the exchange at any given exchange point More than two chromatids can be involved over larger regions In organisms

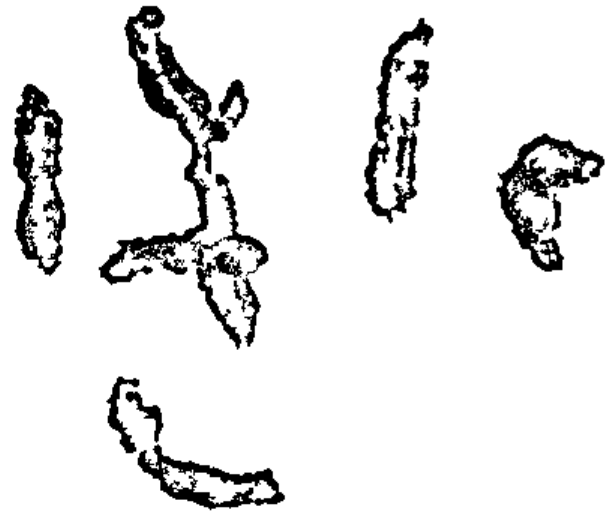


Fig 7 11 Diplotene cell of barley (n=7) (Courtesy of Mrs Christene F Fastnaught McGriff Department of Plant and Soil Science Montana State University Bozeman)

with large chromosomes the two chromatids involved in such a chiasma are seen to cross reciprocally from one homologue to the other (see Fig 4 5) The number of chiasmata per homologous chromosome pair seems to depend on the species and on the length of the chromosomes The longer the chromosome the more chiasmata are present Up to 12 chiasmata have been observed in the long chromosomes of the broad bean *(Vicia faba)* (Swanson 1957) As diplotene progresses the chiasmata seem to move away from the centromere and diminish in number This process is called **chiasma terminalization** (Darlington 1929a) The terminalization process may be complete partial or altogether absent In the last instance the chiasmata are called **localized chiasmata** Especially in large chromosomes terminalization may not be complete One of the main forces during chiasma terminalization seems to be a strong repulsion force at the centromeres (Darlington 1937) As the centromeres move apart the chiasmata slide over the crossover

Fig 7 12 Diplotene cell of a *Triticum* × *Agropyron* derivative with evidence of chiasmata (× 1585) (Schulz Schaeffer unpublished)

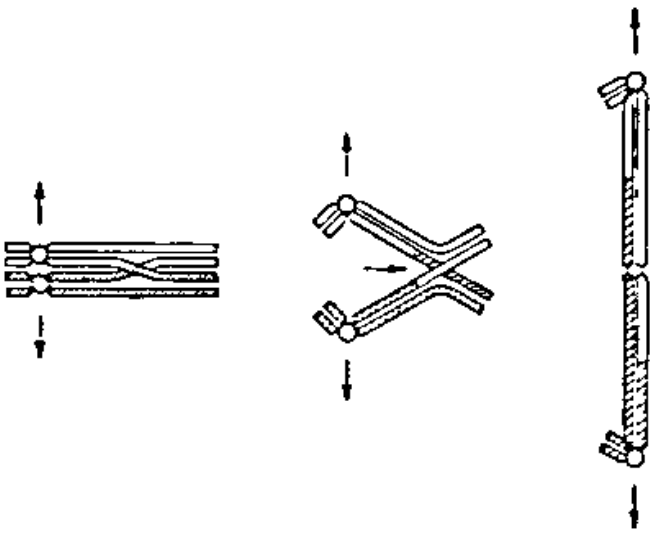


Fig 7 13 Diagrammatic illustration of three successive stages of chiasma terminalization (Rieger et al 1976)

points along the chromatids that are involved in the exchange and toward their distal portions (Fig 7 13) As the chiasmata move toward the chromosome ends they seem to become arrested there forming **endchiasmata** As a consequence an endchiasma is the result of the terminalization of one or more **interstitial chiasmata** It is as if they become locked in at the telomeres without being able to slide over these end structures The purpose of this mechanism is to hold the homologues together until metaphase orientation is completed and extreme tension is exerted on the endchiasmata at which time a special trigger mechanism separates all chromosomes and at the same time ushers in anaphase I The forces that hold the chromosomes together at the end of terminalization are not known as yet

There are three main hypotheses to explain the mechanism of chiasma terminalization

1 Electrostatic hypothesis (Darlington and Dark 1932 Darlington 1937)
2 Coiling hypothesis (Swanson 1942 1957)
3 Elastic chromosome repulsion hypothesis (Östergren 1943)

Darlington s **electrostatic hypothesis** states that two separate repulsion forces are responsible for movement of the chiasmata to the chromosome ends One of these forces **localized repulsion,** is supposed to be the dominating one that repels the centromeres The second force **generalized repulsion** is supposed to be evenly distributed over the entire surface of the chromosomes and tends to force the chromosomes apart The result is a movement of the chiasmata in a distal direction as described before because the greater force exists between the centromeres Darlington based this hypothesis on the observation that in some organisms there is a greater stretching of the centric loop between the two most interstitial chiasmata

Swanson s **coiling hypothesis** questions the reality of Darlington s forces and explains terminalization as being affected by despiralization of the chromosomes During the coiling cycle (see Fig 6 5) the initial coils are small and numerous and as prophase proceeds the coils decrease in number but the gyres of the coils

increase in diameter This theory postulates that the coiling and associated shortening of the chromosomes develops mechanical tension that will force the chiasmata to slide along the chromosomes The advantage of the coiling hypothesis over the electrostatic hypothesis is that the coiling hypothesis can be tested by varying the degree of coiling This has been accomplished by exposing the bivalents to different temperatures Support for the coiling hypothesis came from a comparison of normal and mutant types in the plant *Matthiola incana* (Lesley and Frost, 1927) In the mutant the chromosomes failed to attain their normal state of contraction, and the chiasmata remained at the original interstitial positions In the normal plants the chromosomes shortened and the chiasmata were terminal

The elastic chromosome repulsion hypothesis of Östergren is based on the idea that chiasma movement eliminates the tension created by the chiasmata themselves A chiasma forces the chromosomes out of shape by preventing repulsion which is effective in the adjacent areas of the bivalent The repulsion force tends to push the chiasmata distally since this is the only direction in which relief from tension can be gained

During diplotene the bivalents are generally observed more distinctly because of a widening gap between them, which suggests a repulsion force This is even more evident in the next substage, **diakinesis.**

In some specialized tissues, diplotene can be very much prolonged and can last a year or much longer, not only an hour or two as indicated in Table 7 1 The long duration of this stage in these instances is associated with a specialized function of the cells involved Such prolonged diplotene stages are found in the **primary oocytes** of some vertebrates such as fishes (sharks), amphibians, reptiles, birds, mice, in human beings, and in the **primary spermatocytes** of some insects such as *Drosophila* In these cells the chromosomes acquire a very characteristic appearance They become very diffuse by forming thin threads or loops that are transverse to the main axis of the chromosomes This despiralization makes these chromosomes look like old-fashioned, oil-lamp chimney brushes, and they are therefore called **lamp brush chromosomes** (Section 9 6) These chromosomes also increase enormously in length The purpose of this increase in surface and length is to provide for increased metabolic activity of these chromosomes The loops are believed to be active genetic material such as DNA, which synthesizes messenger RNA that is responsible for protein synthesis in the cells' cytoplasm This causes an enormous growth of the oocytes Oocytes of the frog, *Rana pipiens* increase in size by a factor of 27,000 over a period of three years (Balinsky, 1970) In chicken that factor is 200 (last rapid growth), and in mice 43, and in both cases the growth proceeds at a much faster rate and takes a shorter period for completion In female human beings the diplotene oocytes are already formed by the fifth month of prenatal life Here they remain in diplotene for a period of 12 to 50 years, from the age of sexual maturity of humans to the age when the last eggs are ovulated The functional significance of such a very prolonged diplotene stage is unknown (Swanson et al , 1967) This prolonged diplotene condition is referred to as **dictyotene.**

7 2 5 Diakinesis

If chromosome counts are desirable, then diakinesis may be one of the most ideal stages for this purpose The only disadvantage is the shortness of this stage (see Table 7 1) But the extreme coiling and the seeming repulsion between the bivalents space them all over the cell in squash preparations The restricting nuclear envelope can be ruptured under the pressure of squashing In this respect diakinesis also has the advantage over metaphase I in that the spindle fiber apparatus is not yet attached to the chromosomes This apparatus generally prevents an even spread of chromosomes and keeps them in a bunch

The bivalents have their greatest degree of terminalization and contraction in diakinesis In some cases they become almost spherical configurations (Fig 7 14) If interstitial chiasmata, located close to the centromeres, remain localized, then the bivalents appear like crosses Similar configurations can be formed by **collochores** (Cooper, 1941) Collochores are small conjunctive segments in the regions adjacent to the centromere that are responsible for meiotic chromosome pairing without chiasma formation and for the coherence of such pairing associations until the beginning of anaphase I Cross bivalents observed in certain *Triticum* x *Agropyron* hybrids and their backcross derivatives (Schulz Schaeffer et al , 1971, Schulz-Schaeffer and McNeal, 1977) were interpreted as being formed by collochores (Fig 7 15) There is also good evidence from research in mantids (White, 1938 Hughes Schrader, 1943a, 1943b), lepidoptera (Bauer, 1939), mites (Cooper, 1939), scorpions (Piza, 1939), bugs (Schrader, 1940a, 1941), and flies (Cooper, 1944) that chromosomes can join and hold together during meiosis by mechanisms other than synapsis and chiasmata Further electron-micrographic studies like those that led to the discovery of the synaptonemal complex may shed light on the possible formation of collochores

Other possible chromosome associations in diakinesis are **rod bivalents** and **open ring bivalents** Rieger and Michaelis (1958) defined rod bivalents as pairing associations that had chiasma formation and terminalization in only one chromosome arm in each of the two homologous chromosomes involved (Fig 7 16D) This def

Fig 7 14 Diakinesis cell of barley (n=7) (× 3436) (Schulz Schaefer, unpublished)

Fig 7 15 Photomicrograph of a diakinesis cell of a *Triticum* × *Agropyron* derivative with 25 cross bivalents and 1 univalent (2n = 51) (× 885) (From Schulz Schaeffer et al 1971 Reprinted by permission of Verlag Paul Parey Berlin)

inition could also apply to the more frequent fairly normally occurring phenom enon of **open ring bivalents** (Fig 7 16E) There has to be a reason for the obvious difference in shape between rod bivalents and open ring bivalents A possible explanation would seem to be that rod bivalents have previously synapsed over only a minute distal portion of one arm of each of the two chromosomes involved or that these chromosomes join by organelles similar to the collochores In the case of open ring bivalent formation synapsis and the repulsion following usually cause the bow shape that is also typical for closed ring bivalents and is not expressed in rod bivalents A schematic representation of several possible chromosome configurations in diakinesis is presented in Figure 7 16

The nucleoli usually fuse toward the end of prophase I to form one large nucleolus At the end of diakinesis, the nucleolus begins to disappear

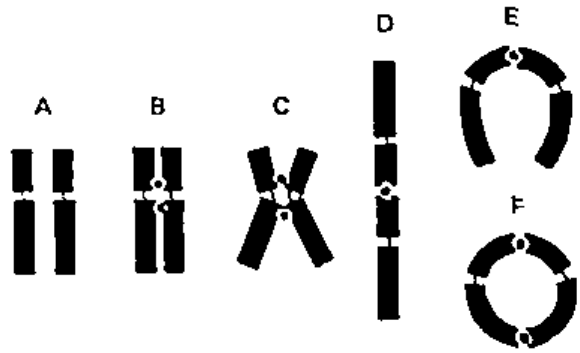


Fig 7 16 A F Schematic representation of chromosome configurations in the diakinesis of *Triticum* × *Agropyron* derivatives Configurations are arranged in a meaningful way to imply tendency for progressive pairing from complete asynapsis (*A*) to normal closed ring bivalents (*F*) (*A*)–Two homologous chromosomes in asynapsis (*B*)–H type cross bivalent (*C*)–Standard type cross bivalent (*D*)–Rod bivalent (*E*)–Open ring bivalent (*F*) Closed ring bivalent (•–collochores and endchiasmata (|–centromeres) (From *Schulz Schaeffer et al 1971 Reprinted by permission of Verlag Paul Parey, Berlin*)

7.3 Metaphase I

Similarly as in mitosis the end of prophase I is also marked by the disappearance of the nuclear envelope and the nucleolus as well as by the division of the centrosome and formation of the spindle Bivalents assemble at the equatorial plate and become oriented with their centromeres poleward Figure 7 17 shows a metaphase I cell of barley in which each of the seven bivalents is clearly visible in the equatorial plate If more than 14 chromosomes are involved, they are not as easily identifiable as in barley An example is shown in Figure 7 18 in which the $2n = 108$ chromosomes of the hexaploid native American rubber plant guayule *(Parthinium argentatum)* are shown in metaphase I

There is a pronounced difference between mitotic metaphase and metaphase I of meiosis (Fig 7 19) In mitosis, the sister chromatids are held together by functionally undivided centromeres (C), which are located on the equatorial plate exactly halfway between the poles In meiosis, the two centromeres (C) of the homologues are not located on the equatorial plate but are oriented in the long axis of the spindle equidistant from the equator, while the endchiasmata (E-C) are located in the equatorial plate The coordinated arrangement of all tetrad chromosomes on the equatorial plate in the described manner is called **coorientation** (Darlington, 1937) An equilibrium is established at the equatorial plate after all tetrad chromosomes (bivalents) have attained this position, until the chromosomes yield to the tension exerted on them originating from the two opposite poles via the spindle fibers

This final arrangement of the bivalents on the equatorial plate also has some genetic consequences Just as crossing over during zygotene and pachytene provides for recombination of paternal and maternal genes on the chromosomes as discussed so does the coorientation of the bivalent on the equatorial plate provide for recombination of paternal and maternal chromosomes during metaphase I In general the position of each chromosome of a bivalent with respect to the poles seems to be at random (Fig 7 20, **random assortment**) There may be exceptions in which preferential segregation is involved (Section 17 5) The random orientation of the bivalents on the equatorial plate determines the **meiotic segregation** and distribu tion of the paternal and maternal chromosomes to the daughter cells of the first

Fig 7 17 Metaphase I in barley ($n=7$) (Courtesy of Mrs Christine E Fastnaught McGriff, Depart ment of Plant and Soil Science Montana State University, Bozeman)

Fig 7 18 Metaphase I of hexaploid guayule *(Parthenium argentatum)* (n = 54) (× 1438) (Courtesy of Dr Duane Johnson Department of Plant Science University of Arizona Tucson)

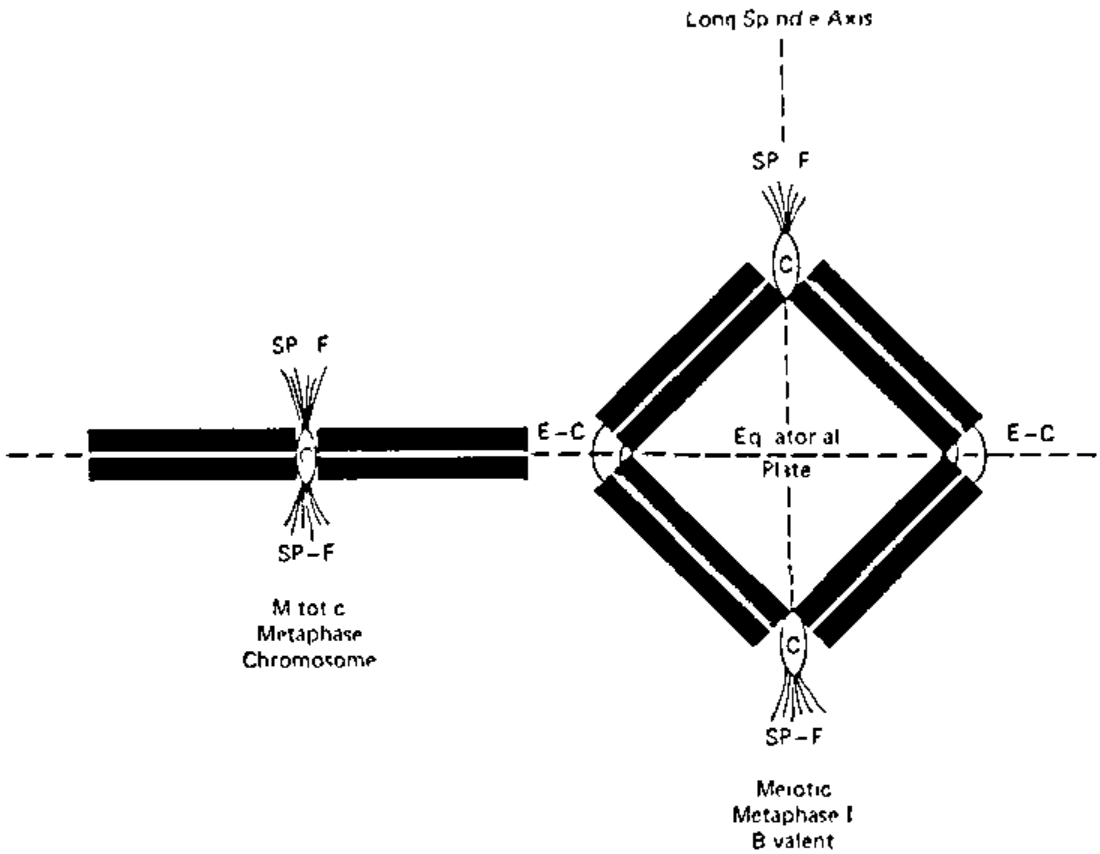


Fig 7 19 Illustration of the differences in chromosome orientation between mitotic metaphase and metaphase I of meiosis SP-F = spindle fiber attachment E-C = end-chiasma C = Centromeres

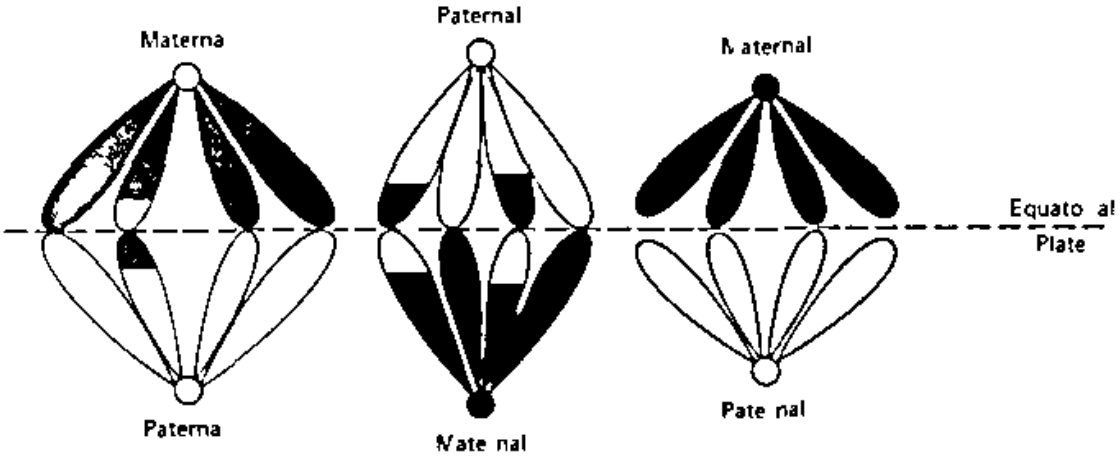


Fig 7 20 Diagrammatic representation of random assortment of homologous chromosomes at the equatorial plate of a metaphase I nucleus

meiotic division Meiotic segregation is the substance of Mendel s second law (See Chapter 1) the law of independent assortment Mendel s law made statements concerning gene segregation while meiotic segregation deals with gene blocks Mendel s law agrees with the assumption that two factor pairs under consideration are located on different bivalents The discovery of linkage brought cytological and genetical discoveries into alignment in that gene blocks or linkage groups were now accounted for

7 4 Anaphase I

During anaphase I the tetrad chromosomes separate into dyad chromosomes (Fig 7 21) as the two cooriented centromeres move toward opposite poles (Fig 7 2) The difference between anaphase I (A I) and mitotic anaphase (or A II) is best exemplified in Fig 7 1 A typical meiotic anaphase I always has four chromosome arms dangling behind the centromere while a mitotic anaphase has only two such arms showing The reasons for this difference is the fact that an anaphase I dyad chromosome consists of two chromatids, while a mitotic anaphase chromosome is really a single chromatid The four arms of an anaphase I dyad chromosome do not stick closely together but diverge as if they are mutually repelling each other Anaphase I is shorter in duration than metaphase I (see Table 7 1) The only real function of this stage is to evenly distribute the partners of homologous chromosome pairs to the daughter nuclei with the result of a reduction by half the num*ber in each resulting nucleus The original* **somatic chromosome number** *(2n) is* reduced to a **gametic chromosome number** (n) These two symbols are very often used in reports of chromosome numbers If a species is investigated in somatic tissue (mitosis), the chromosome count is reported as 2n (e g 2n = 28) If a count is made in gametogenesis (meiosis) the report is made with an n number (e g n = 14) This understanding enables the cytologist to quickly identify the nature a particular chromosome report Very often ploidy levels are erroneously reported with n numbers But the number reserved for ploidy levels is the x number or

Fig 7 21 Anaphase I of barley (n = 7) (× 1453) (Schulz Schaeffer unpublished)

basic genome number (x, 2x, 4x, 6x, etc) (see also Chapter 2) Well known summaries of chromosome numbers have been published for animals by Makino (1951) and for plants by Darlington and Fedorov (Darlington and Janaki Ammal 1945, Darlington and Wylie, 1955, Fedorov, 1974) Makino's report has data on some 2,800 animal species, and Fedorov's has data on 35 000 plant species
Each anaphase I dyad chromosome has two chromatids that remain joined at the centromere until anaphase II

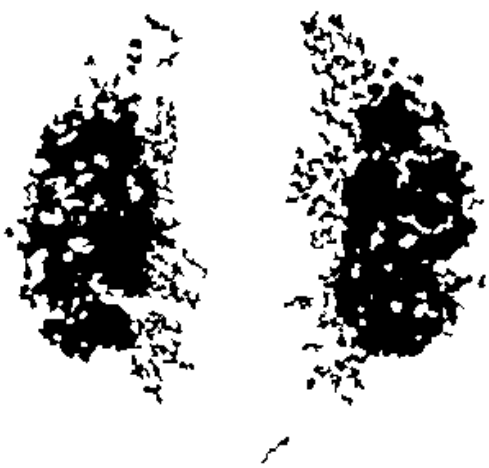
7.5 Telophase I and Interkinesis

Telophase I is similar to mitotic telophase in that the chromosomes assemble at the poles (Fig 7 22) But since the following interphase (called **interkinesis**) is different from normal interphase, telophase I can also be different in several respects, depending on the organism During interkinesis, which is a short stage, the chromosomes do not synthesize new DNA and consequently there is no reduplication The chromosomes are already prepared for the second division in that each of them consists of two chromatids only held together by a centromere Therefore, despiralization, uncoiling, and hydration of chromosomes are not necessary As a matter of fact, in some species following the disappearance of the spindle, the chromosomes orient themselves at the poles and pass directly to the equatorial plate of the second division (M II) This has been reported for *Trillium* (Swanson 1957) and certain members of the Odonata (Cohn, 1969) In this case the coiling of the chromosomes is retained throughout interkinesis In other instances the chromosomes become partially uncoiled during interkinesis, and nuclear envelopes form This

Fig 7 22 Telophase I of *Bromus inerm s* (n=28) (× 1077) (Schulz Schaeffer unpubl shed)

k nd of interkines s has been observed in *Tradescantia Zea mays* and grasshopper (Swanson 1957) *Figure 7 23 shows an interkinesis cell of Agropyron intermed um*

In other organisms there is no cytokinesis after the f rst meiot c division as reported for *Paeon a* where no walls are formed at interkinesis (Swanson 1957) Cytokinesis is postponed until after the second me ot c division and this process is referred to as **quadripartitioning** In contrast the normal process as found in many other plants where a cell plate forms between the telophase nuclei of the f rst division is called **bipartitioning**



F g 7 23 Interkines s of *Agropyron intermed um* (n=21) (× 1239) (Schulz Schaeffer unpubl shed)

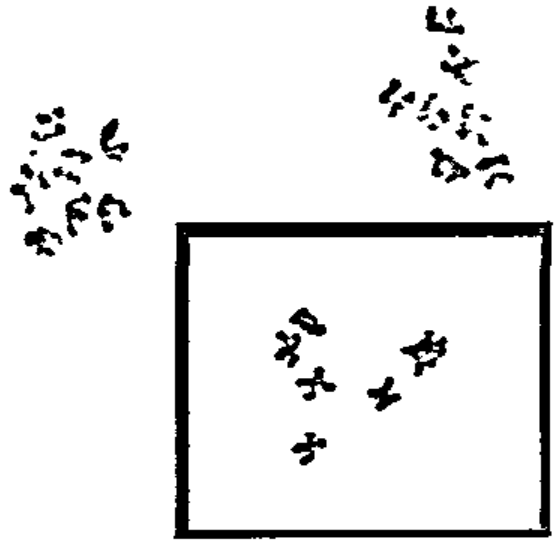


Fig 7.24 Prophase II in barley (n=7) (× 1093) Insert single prophase II cell showing nucleolus (Schulz Schaeffer unpublished)

7 6 Prophase II

The second meiotic division in many respects is very similar to a mitotic division Second prophase differs in appearance from first prophase in that the sister chromatids of each dyad chromosome show a very striking repulsion so that the chromatid arms are widely separated from each other (Fig 7.24) This makes the dyad chromosomes look like crosses The shortness of the only partially uncoiled chromosomes makes it possible to view each prophase II chromosome individually, and chromosome counts can sometimes be made

Fig 7.25 Metaphase II cell of *Agropyron intermedium* (n=21) (× 1333) (Schulz Schaeffer, unpublished)

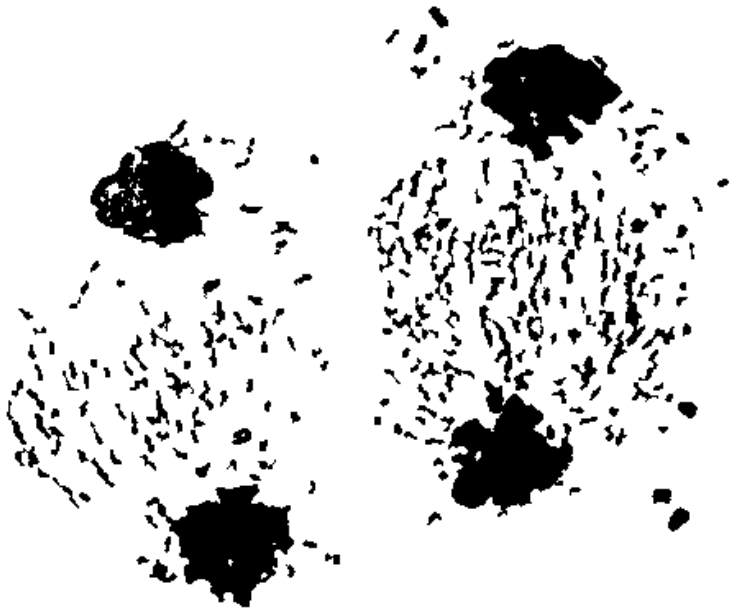


Fig 7 26 Telophase II in *Agropyron intermedium* (n=21) (× 1554) (Schulz Schaeffer unpublished)

The genetic constitution of the two sister chromatids in the second meiotic division depends on the extent of crossing over during the prophase of the first division In this respect the second meiotic division differs from a mitotic division that is strictly equational Figure 7 2 illustrates that prophase II dyads can be partly maternal and partly paternal in genetic makeup In this respect the second meiotic division really completes the process of **genetic recombination** that was started with crossing over in prophase I Crossing over of chromatid segments and random distribution of maternal and paternal chromosome segments during the first and second meiotic divisions are together the agents of genetic recombination in meiosis

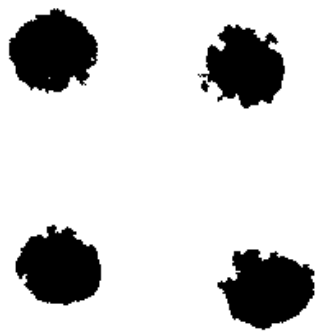


Fig 7 27 Radial quartet in barley (Schulz Schaeffer unpublished)

Fig 7 28 Microspore of *Triticum* × *Agropyron* derivative (Schulz Schaeffer unpublished)

7.7 Metaphase, Anaphase, and Telophase II

The centromeres of the dyad chromosomes situate on the equatorial plate as in an ordinary somatic division In higher plants, the two adjacent cells, separated only by a cell wall, generally go through a synchronized procedure of chromosome congression, orientation, and distribution (see Section 6 4) The two equatorial plates are lined up across the separating cell wall Figure 7 25 shows a metaphase II cell of *Agropyron intermedium* As the centromeres become functionally double, the **monad chromosomes** (Fig 7 2) move toward the four poles of the **duet cells** of **microsporogenesis** and form a telphase II cell (Fig 7 26) As the cell walls form at the end of telophase II, the so-called **radial quartet cells** form The quartets are the four adherent cells resulting from the two meiotic divisions in microsporogenesis (Fig 7 27) The quartets then differentiate into four haploid microspores (Fig 7 28), which are the endproducts of microsporogenesis as described in the next chapter The two meiotic divisions in **spermatogenesis** also lead to four daughter cells (**spermatids**) that subsequently differentiate into four sperms **Oogenesis** and megasporogenesis are different (unequal) in that only one large haploid egg and three small **polar bodies** in animals and only one large megaspore in plants are produced This completes the meiotic cycle in plants and animals

Chapter 8
Chromosomes During Sexual Reproduction

In this chapter special attention is paid to the importance of chromatin and chromosomes in reproduction. However, in order to understand what happens at the chromosomal level, the whole cell is considered carefully. In the last chapter we stated that the processes of genetic recombination in meiosis are: (1) crossing over of chromatid segments during prophase I and (2) random distribution of maternal and paternal chromosome segments during the first and second meiotic divisions. But meiosis is only one part of sexual reproduction. During the life cycles of **haplontic, diplontic,** and **diplo-haplontic** organisms there is a regular alternation between meiosis and fertilization. The second part of genetic recombination is the union of paternal and maternal gametes during syngamy.

In order to understand the differences in the life cycles of various plants and animals, the *newly introduced* terms **haplontic, diplontic,** and **diplo-haplontic** should now be explained (Cook, 1965). **Haplonts** are common in most unicellular or filamentous algae and protozoa. These are organisms in which the **haplophase** is more prominent than the **diplophase** (Renner, 1916). The haplophase is the **functionally haploid** period (n) during a particular life cycle lasting from meiosis to fertilization, while the diplophase is the **functionally diploid** period (2n) of a life cycle that spans from fertilization to the beginning of meiosis. The term **diploid** is used here in a wider sense since it really designates only organisms with two basic genomes (2x) (Section 2.1.1). This usage of **functional diploidy** is further explained in Section 15.2. In the haplonts, only the zygote is diploid (Fig. 8.1) and in some species it becomes a resistant spore, which guarantees the survival of the species under difficult conditions. The mature individuals in this life cycle, which in multicellular organisms differentiate by mitotic divisions, are functionally haploid (n).

***Diplonts** are found in humans, higher animals, and in some algae. They are organisms in which the diplophase is more prominent than the haplophase.* In diplonts the products of meiosis function directly as gametes. This is the regular life cycle of all multicellular animals. Only the gametes are haploid in this type of life cycle (Fig. 8.2). The mature individuals are produced by mitotic division and differentiation and are functionally diploid (2n). In contrast to diplo-haplontic organisms, the diplonts do not have alternation of generations but only alternation of nuclear phases (n and 2n).

Diplo-haplonts are typical in higher plants and in many algae and fungi. As early as 1851 the concept of **alternating generations** for such organisms had been devel-

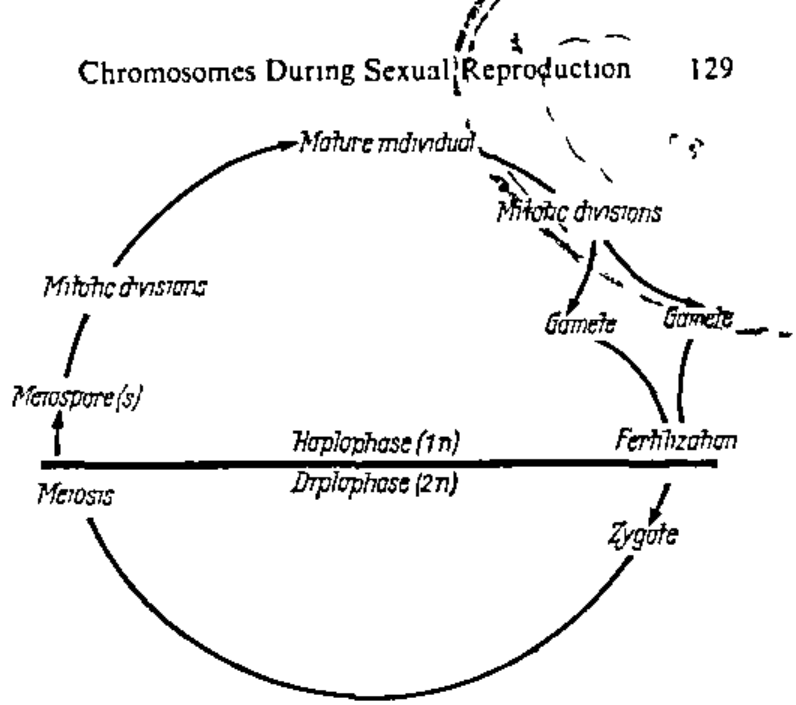


Fig 8 1 Diagram of the life cycle of haplonts that are common in most unicellular filamentous algae and protozoa (From Cook 1965)

oped by Hofmeister He demonstrated that the life cycle of a typical plant consists of two unique generations a spore-bearing (**sporophyte**, 2n) and a gamete bearing (**gametophyte**, *n*) (*Fig* 8 3) The sporophyte of higher plants makes up the more prominent generation Meiosis in diplo haplonts does not immediately produce gametes as in higher animals, instead, a parasitic structure, which in turn produces the gametes, is inserted as an alternating generation in higher plants This parasitic structure is called the gametophyte The relationship between the sporophytic and gametophytic generations varies depending on plant groups In the **Spermatophyta** (seed plants) the sporophyte is dominant and independent Its life duration is from one to several years The gametophyte is extremely small and

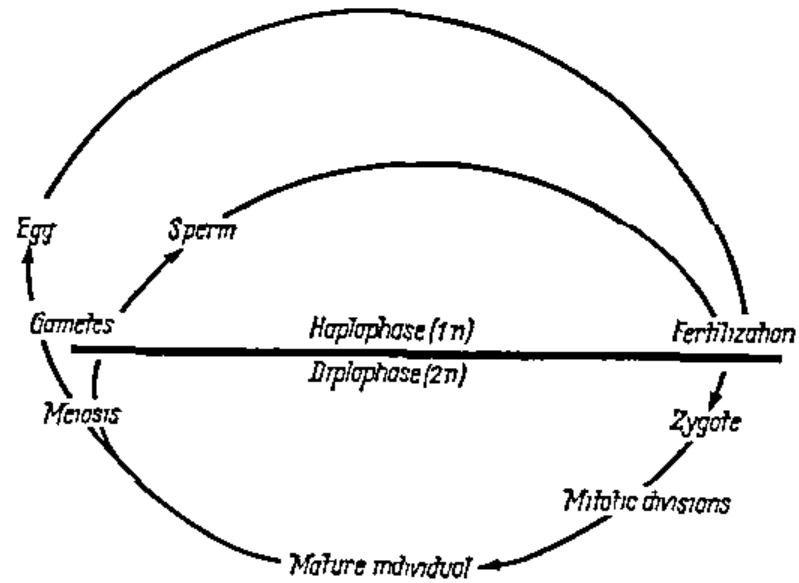


Fig 8 2 Diagram of the life cycle of diplonts that are representative of man, higher animals and some algae (From Cook 1965)

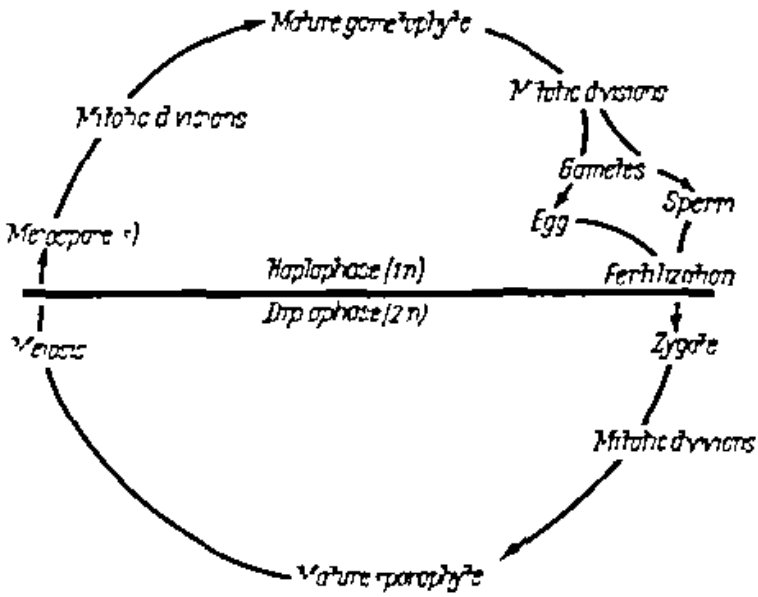


Fig 8 3 Diagram of the life cycle of diplo-haplonts that are typical of higher plants and many algae and fungi (From Cook, 1965)

parasitic on the sporophyte, as mentioned, and it lives only from a few days to a few weeks In the **Pteridophyta** (ferns and related plants), the sporophyte is dominant, vegetatively independent, and often perennial The gametophyte, though small, is independent of the sporophyte and lives a few weeks or longer In the **Bryophyta** (mosses and liverworts), the sporophyte is partially parasitic on the gametophyte and lives a few weeks A gametophyte is dominant, vegetatively independent, and lives one to several years The diagram in Fig 8 4 illustrates these relationships between Spermatophyta, Pteridophyta, and Bryophyta Single lines represent the functionally haploid (n) gametophyte generation and double lines represent the functionally diploid (2n) sporophyte generation The lengths of the lines generalize the relative prominence and length of the two generations in the life cycle Generations represented by unbroken lines are independent and

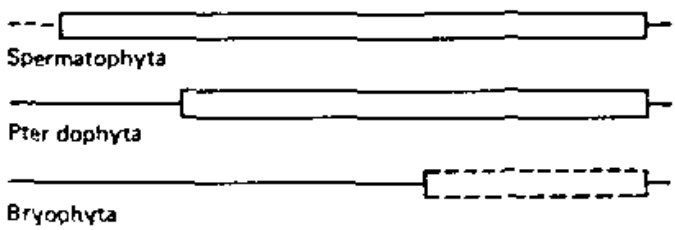


Fig 8 4 Diagrammatic representation of the relationsbip between gametophyte and sporophyte generations in plants
Single lines–haploid gametophyte generation
Double lines–diploid sporophyte generation
Length of lines approximate relative prominence of gametophyte and sporophyte generations
Unbroken lines–independent generations
Broken lines–parasitic on other generation (From Alexander, 1954 Redrawn by permission of Barnes and Noble, Inc , New York)

those shown by broken lines are parasitic on the other generation in the life cycle (Alexander, 1954)

8 1 Sexual Reproduction in Plants

Reproduction in plants can be subdivided into sporogenesis, gametogenesis, and syngamy The example for reproduction in plants will be taken from the Spermatophyta, which make up the dominant part of our vegetation As mentioned above they exemplify a diplo-haplontic life cycle The diplophase is called the sporophyte The sporophyte differentiates two kinds of tissues that later will develop the **meiospores.** The tissue that leads to **microspores** is called the **microsporangium** and is located in the **anthers** The tissue that eventually will produce megaspores is called the **megasporangium** and is located in the **ovary**

8 1 1 Microsporogenesis and Spermatogenesis

An illustration of microsporogenesis and spermatogenesis in angiosperms is shown in Fig 8 5 **Microsporogenesis** is the process of microspore formation that leads to the first cell of the male **gametophyte** generation Microsporogenesis takes place in the microsporangium A typical anther of a phanerogam (Spermatophyta) has four elongated microsporangia that develop into **microsporocytes** or **pollen mother cells (PMC's)** These cells enlarge and go through meiosis, as described in Chapter 7 A microsporocyte that goes through meiosis is also referred to as a **meiocyte.** The end product of microsporogenesis is a **radial quartet cell.** The radial quartet cells each separate into four haploid **microspores** with one nucleus each Each of these microspores may develop into a pollen grain **(spermatogenesis)**

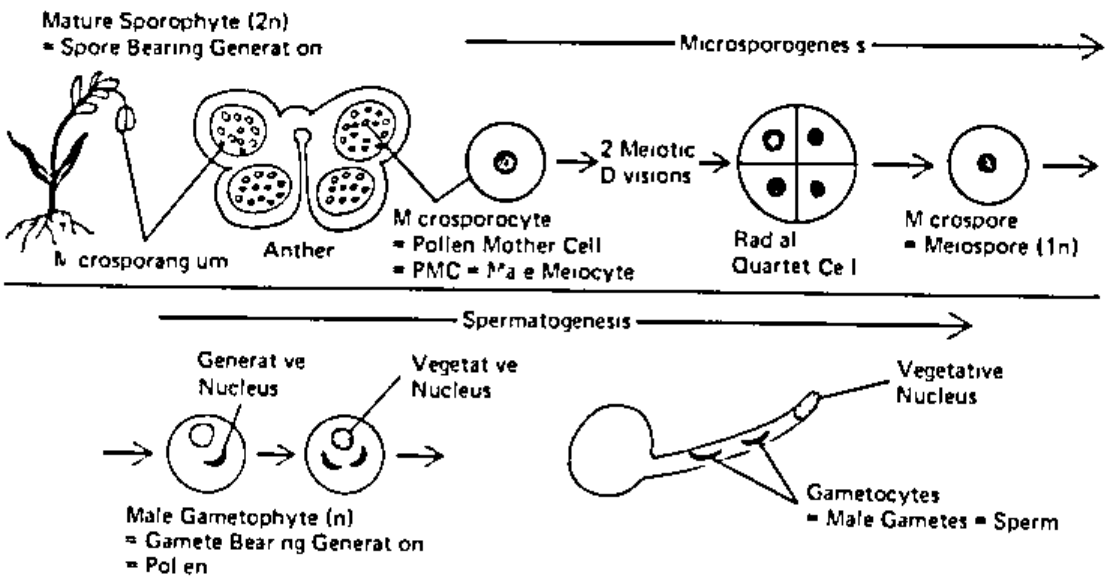


Fig 8 5 Microsporogenesis and spermatogenesis in angiosperms

Between the microspore stage and the first pollen mitosis there is a rest period that varies from a few hours to several months (Dahlgren, 1915, Finn, 1937) The developing pollen grain or male gametophyte enlarges during this period either because of an increase in the amount of cytoplasm (Sax and Edmonds, 1933), or because of the formation of vacuoles in the cytoplasm (Steffen 1963), or because of both of these reasons together Naturally, there is also an increase in the amount of DNA before pollen mitosis (Bryan, 1951) because during synthesis the chromosomes replicate

Pollen mitosis is an ideal stage for chromosome analysis The chromosomes are reduced by half (n) which facilitates the count and the spread of the chromosomes in organisms with high chromosome numbers They are also less contracted than during the two meiotic divisions, which makes it easier to discern their centromere positions

The nuclei that result from the first pollen mitosis differentiate into a **generative** nucleus and a **vegetative** or **tube nucleus.** The male gametophyte now has two nuclei that were produced by **karyokinesis** (nuclear division) without the event of **cytokinesis** (cytoplasmic division)

The two nuclei of the male gametophyte usually differ in shape The generative nucleus is densely compacted and often is crescent shaped, while the vegetative nucleus is larger and spherical and less densely stained These nuclei are often considered to be **protoplasts** or cells, but they definitely lack cell walls Electron microscope studies have shown a clearly defined double membrane around the cytoplasm of the generative nucleus but no wall as earlier reported from light microscope studies (Bopp-Hassenkamp, 1960) The DNA content of the two morphologically different nuclei seems to be similar (Swift, 1950; Bryan, 1951, Ogur et al , 1951)

A **second pollen** mitosis leads to the production of a **mature male gametophyte** or **pollen** This mitosis occurs in the generative nucleus of the pollen and leads to the formation of the two **male gametes** or sperms These are also referred to as the **male gametocytes** The time of the second pollen division varies according to species In many grasses the three nuclei are present before pollen tube formation In lily, second pollen mitosis happens in the pollen tube when the sperm passes through it down the style on its way to the micropylar opening of the ovule If pollen mitosis takes place in the pollen tube, the metaphase chromosomes form rows parallel to the axis of the pollen tube (Fig 8 6) The vegetative nucleus seems to be responsible for the growth of the pollen tube and is the one in the tip of the pollen tube trailed by the two sperms (Fig 8 5) The vegetative nucleus and the sperms are believed to be passively transported through the streaming cytoplasm *in the pollen tube (Navashin et al , 1959) The sperms are now ready for syngamy*

8 1 2 Megasporogenesis and Syngamy

An illustration of megasporogenesis and syngamy is shown in Fig 8 7 **Megasporogenesis** is the process of megaspore formation in the **megasporangium** or **ovule** The megasporangium consists of the **nucellus** and of one or two **integuments** Enclosed in the nucellus is the **megasporocyte** or **embryo sac mother cell** (EMC)

Fig 8 6 Pollen mitosis in the pollen tube of colchicine treated tetraploid *Polygonatum commutatum* (n = 2x = 20) (Redrawn by permission from Colchicine—in Agriculture Medicine, Biology and Chemistry by O J Eigsti and P Dustin, Jr © 1955 by The Iowa University Press Iowa 50010)

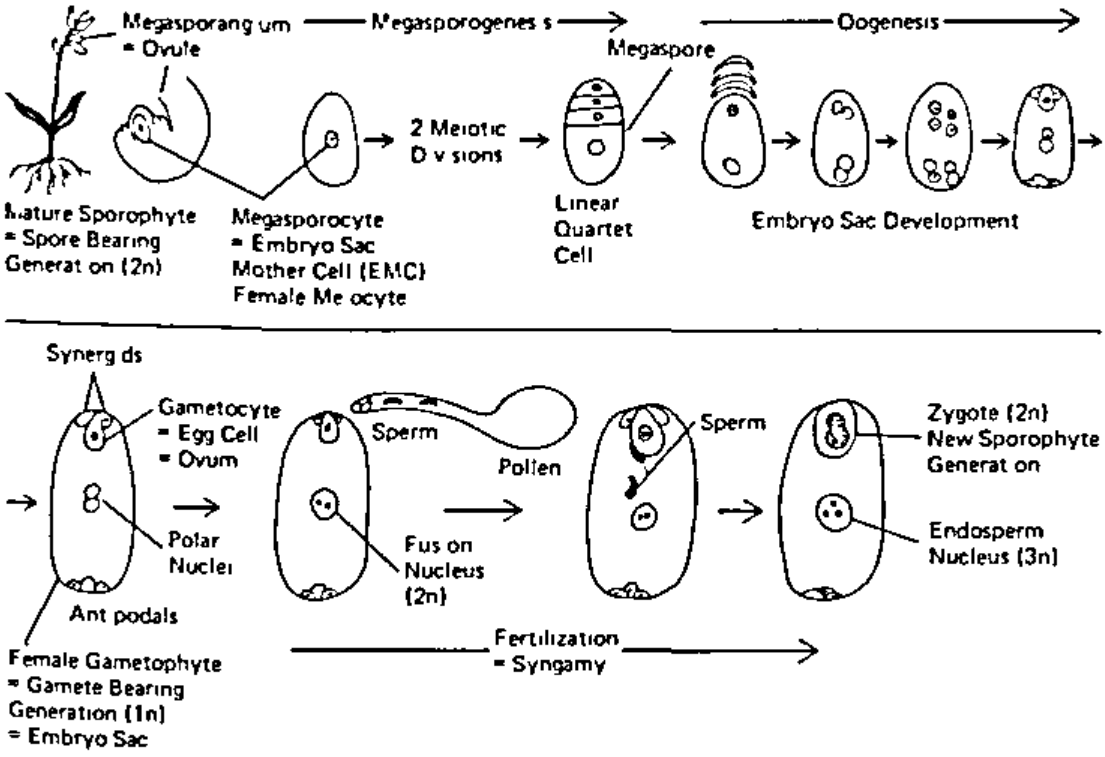


Fig 8 7 Megasporogenesis and syngamy in angiosperms

Each ovule contains only one megasporocyte The megasporocyte divides by meiosis to form a **linear quartet cell,** which is a row of four cells each of which is a potential megaspore Three of these cells degenerate but the fourth enlarges and forms the large **megaspore** The megaspore typically develops into the **embryo sac** through three mitotic divisions Eight nuclei are formed by these divisions that organize into the **egg apparatus,** two **polar nuclei** and three **antipodals** (Fig 8 7) The embryo sac thus becomes the **female gametophyte** (n) or *the gamete bearing generation The egg apparatus consists of the two outer* **synergids** and the egg nucleus in the center The egg nucleus becomes the **egg cell female gametocyte,** or **female gamete** The two polar nuclei often fuse and become a diploid **fusion nucleus** (2n)

Syngamy is preceded by the landing of the pollen grain on the **stigma** of the female portion of the flower which consists of stigma **style,** and **ovary** (**pistil**). The pollen grain germinates on the stigma The time for germination is variable For instance germination takes three minutes in *Reseda* (Eigsti 1937) and five minutes in *Zea mays* (Randolph 1936) The pollen forms a tube that passes down the style and reaches the opening of the ovule called **micropyle** After the two sperm enter at the micropyle one fuses with the egg cell to form the zygote (2n) and the other *fuses with the fusion nucleus to form the* ***endosperm nucleus*** *(3n) in what is often* referred to as **double fertilization** The time from pollen germination to fertilization varies according to species In general it takes 12 to 48 hours (Maheshwari 1949) The zygote is the first cell of the new sporophyte (2n) which by mitotic cell division develops and differentiates into the mature sporophyte This completes the life cycle of a typical diplo-haplont with its characteristic alternating generations (2n 1n)

8 2 Sexual Reproduction in Animals

In higher animals sexual reproduction consists of **gametogenesis** and **syngamy** *Higher animals are diplonts in which the diplophase is more prominent than the* haplophase The fertilized diploid ovum or zygote divides and differentiates by regular mitosis to form the adult mature animal body a portion of which differentiates into the **germ line** (Weismann 1885) The germ line is a group of cells that early during the development of an animal organism are separated from somatic cells as potential gamete forming cells Gamete formation or gametogenesis takes place in the testes of the male and in the ovary of the female

8 2 1 Spermatogenesis

Gametogenesis in the male animal is called **spermatogenesis** The testes contains the immature potential germ cells called **primary spermatogonia** By rapid mitotic multiplication they produce the so-called **secondary spermatogonia**

The chromosomes in spermatogonial mitosis differ in shape and size from normal mitotic chromosomes They show a spiral configuration and are very much contracted (Fig 8 8) Sasaki and Makino (1965) observed that they were extremely fragile with a tendency to break and scatter A fairly high degree of **polyploidy** has been observed in spermatogonia of humans (Sasaki 1964 Sasaki and Makino

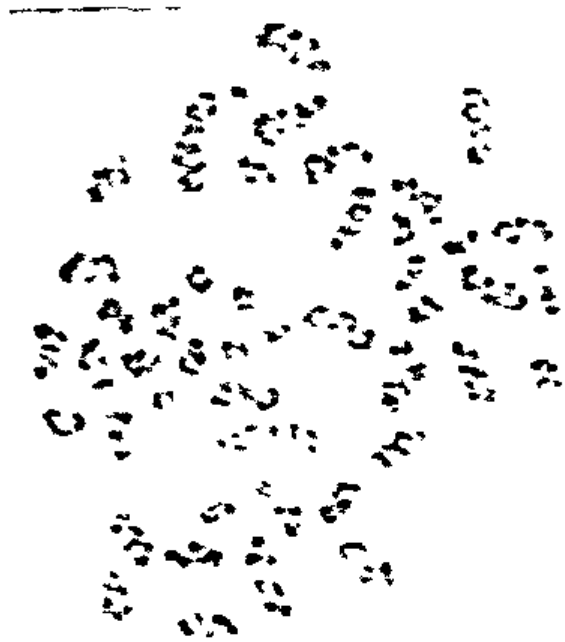


Fig. 8.8 Human chromosomes in spermatogonial mitosis. (× 1600) (From Sasaki and Makino, 1965)

1965, Kyessler 1966, McIlree et al., 1966). McIlree et al. found from 0% to 25% polyploid cells in different individuals, and Sasaki reported an average of 7% to 8.7%. Kyessler's (1970) observations are in agreement with the above percentages. In the vertebrates, the spermatogonia are found next to the basal membranes of the seminiferous tubules (Fig. 8.9). While part of the spermatogonia remain in this

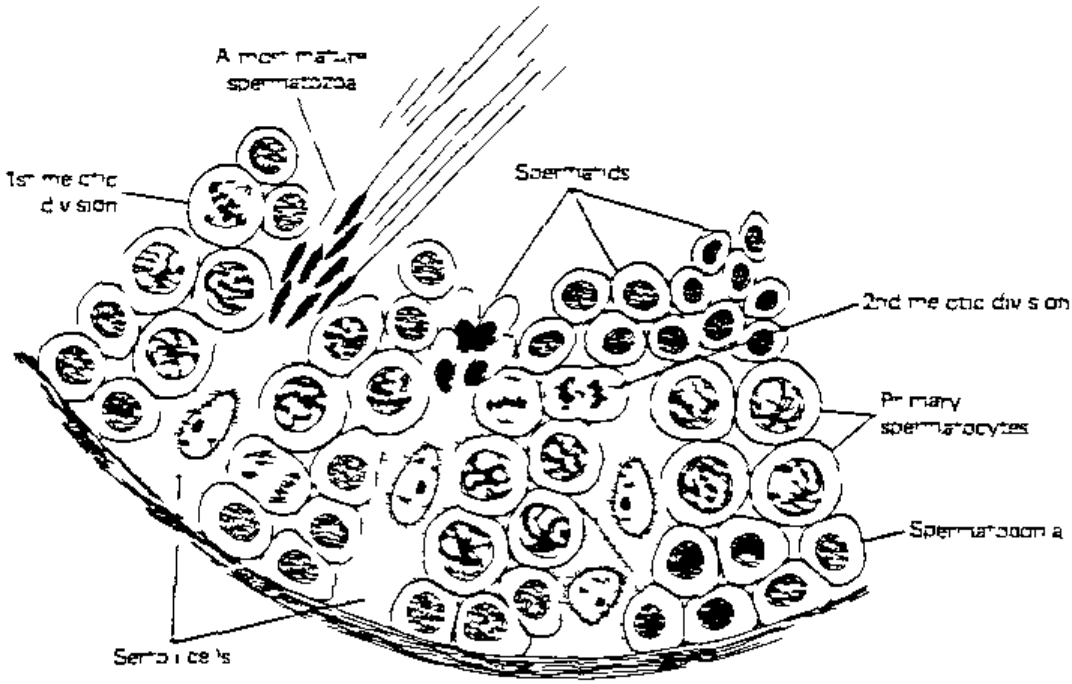


Fig. 8.9 Diagram of part of a seminiferous tubule in a mammal. The spermatogonia are found next to the basal membranes of the seminiferous tubules. (From Balinsky, 1970. Redrawn by permission of W. B. Saunders Co., Philadelphia)

condition and form a source of new sex cells throughout the reproductive life of the animal, some of the cells produced move toward the lumen of the tubule, and enter into the growth phase of spermatogenesis and are then called **primary spermatocytes.** The growth of the spermatocytes is limited, but as a result they become about twice as large in volume as the spermatogonia (Balinsky, 1970) The chromosomes also associate in pairs during this period The primary spermatocytes undergo the first meiotic division and become **secondary spermatocytes.** After the second meiotic division, the **spermatids** are formed The spermatids are haploid sex cells but they are still not capable of functioning as male gametes, which are the so-called **spermatozoa** or **sperm.**

8 2 2 Spermiogenesis

The process of differentiation from an immature spermatid to a mature spermatozoon is called **spermiogenesis.** Spermiogenesis is a striking metamorphosis and involves a very radical change After the second meiotic division, the nucleus of the spermatid goes into a typical interphase forming dispersed chromatin The cytoplasm of a spermatid contains all the inclusions that a normal cell usually has Among them are the mitochondria, the centrioles, and the **Golgi apparatus.** These organelles are instrumental during the development from a spermatid to a spermatozoon One of the most important changes in the morphology during spermiogenesis is the reduction in cytoplasmic material and the condensation and elongation of the nucleus

It seems that the cell tries to eliminate all extra material that is not of importance in motility The main function of the mature spermatozoon is the transportation of the male genetic material to the female egg The spermatozoon differentiates the **acrosome** so that it can penetrate the egg membrane and enter the egg's cytoplasm The acrosome is derived from the Golgi apparatus In the early spermatid, the Golgi apparatus consists of the typical flattened membrane bound sacs, called **cisternae** As the development of the spermatid proceeds, vacuoles develop in the Golgi apparatus Within the vacuoles, small dense bodies appear that are called **proacrosomal granules** (Fig 8 10) The smaller vacuoles coalesce into a larger one, and the granules they contain fuse into one The vacuole and the Golgi apparatus now approach the tip of the elongating nucleus As the Golgi apparatus moves toward the nucleus, the vacuole containing the granule actually moves toward the edge of the Golgi apparatus and attaches to the nuclear envelope (Fig 8 10C) The proacrosomal granule now increases in size as small vacuoles continue to arise from the Golgi apparatus and coalesce with the large vacuole or vesicle, thus adding more proacrosomal material The granule thus becomes the acrosomal granule As the development proceeds further, the vesicle loses its liquid content and becomes completely filled with granule substance (Burgos and Fawcett, 1955)

The next step in the development of the spermatozoon is the formation of its **middle piece** (Fig 8 11B) This part eventually contains the base of the flagellum and the mitochondria, which will serve as a power plant supplying the flagellum with energy This formation of the middle piece begins with the movement of the two centrioles to a place just behind the nucleus opposite the acrosome One of them, the **proximal centriole,** becomes located in a depression of the nucleus The other

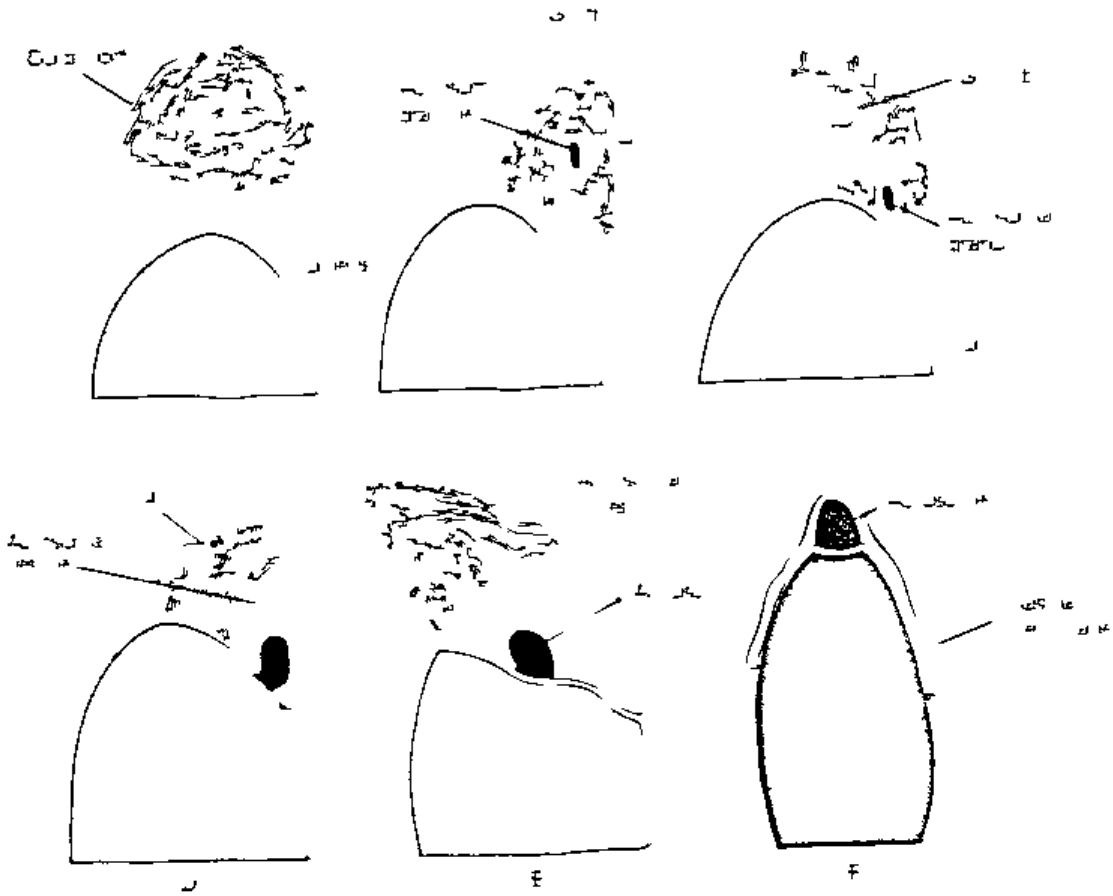


Fig. 8.10 Sequence of stages in the formation of the acrosome and head cap from the Golgi complex during spermatogenesis in the cat. (From Burgos and Fawcett, 1955. Redrawn by permission of the Rockefeller University Press, New York)

one, the **distal centriole**, lines up right behind it at a right angle and coincides with the longitudinal axis of the spermatozoon (Fig. 8.11A). The distal centriole is responsible for the development of the **axial filament** of the flagellum. Mitochondria aggregate around the axial filament. In mammals the mitochondria, which become concentrated in the middle piece from other parts of the cell, lose their individuality and form a **mitochondrial spiral** that winds around the axial filament. At the end of the middle piece, between the middle piece and the tail, is the dense so-called **ring centriole** with unknown function. The name *centriole* is misleading since its fine structure does not resemble a centriole.

The tail or **flagellum** (Fig. 8.11B) is usually the longest part of the spermatozoon. It enables the sperm to swim. Its main part is the axial filament that continues into it from the middle piece. Its fine structure reveals ten pairs of longitudinal fibers, one in the middle and nine surrounding it in a ring.

The final structure of the mature spermatozoon, as shown in Fig. 8.11, contains **head**, **middle piece**, and **tail**. The head consists of the acrosome and an elongated nucleus. The middle piece is composed of the proximal centriole (the distal centriole often disintegrates), the axial filament, and the mitochondrial spiral. The tail is composed mainly of the axial filament.

As seen in Fig. 8.11, the sperm has lost most of the cytoplasm during maturation.

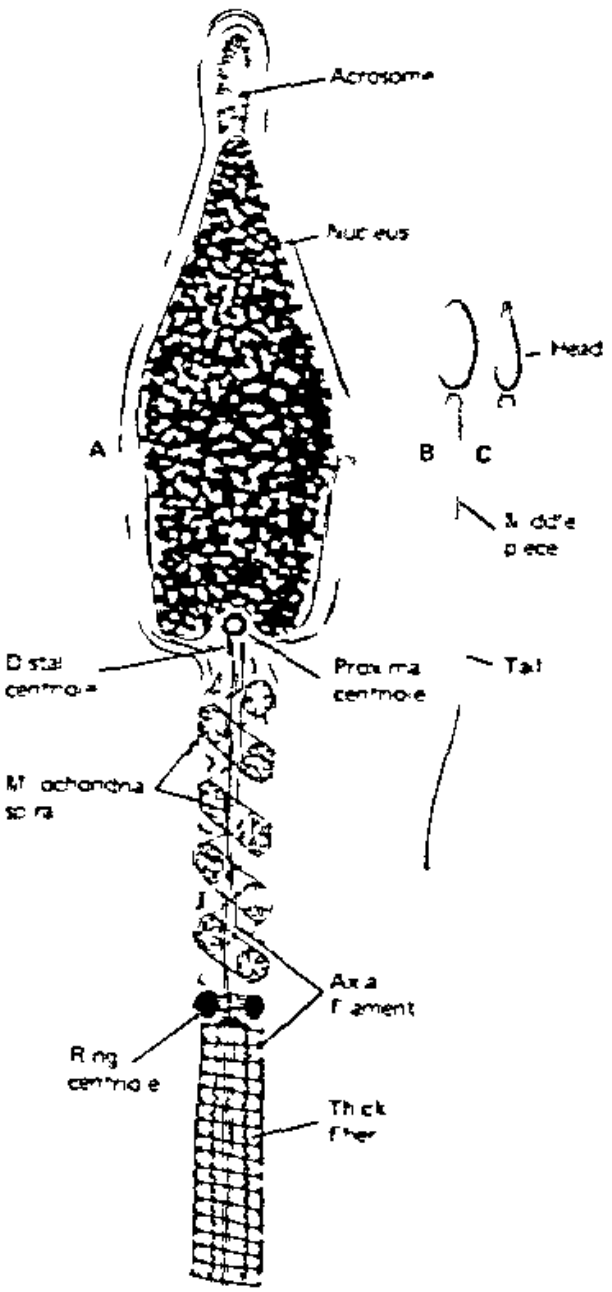


Fig. 8.11A–C. Diagram of a mammalian spermatozoon. (*A*) Detailed diagram as seen under the electron microscope (*B, C*) Diagram of spermatozoon as seen in the light microscope. The sperm head is seen from the flattened side in *B* and from the narrow side in *C* (From Balinsky 1970. Redrawn by permission of W. B. Saunders, Co., Philadelphia)

Only the plasma membrane remains as a sheath around the mature sperm. This means that very little male cytoplasm is transferred to the egg during fertilization. This may be the reason for so-called **maternal effects** that can be caused by extrachromsomal genetic factors in the female cytoplasm. They are thought to be transmitted through the egg but not to be controlled by the genes of the developing embryo (see Chapter 20).

As pointed out at the beginning, the main concern in this chapter is the focus on the chromosomes or the chromatin (DNA) during sexual reproduction. But in order to fully understand what happens in the nucleus, some surrounding structures had to be considered also.

DuPraw (1970) writes that the sperm of animals and some plant cells are highly specialized as motile carriers of the species' haploid DNA component. Associated

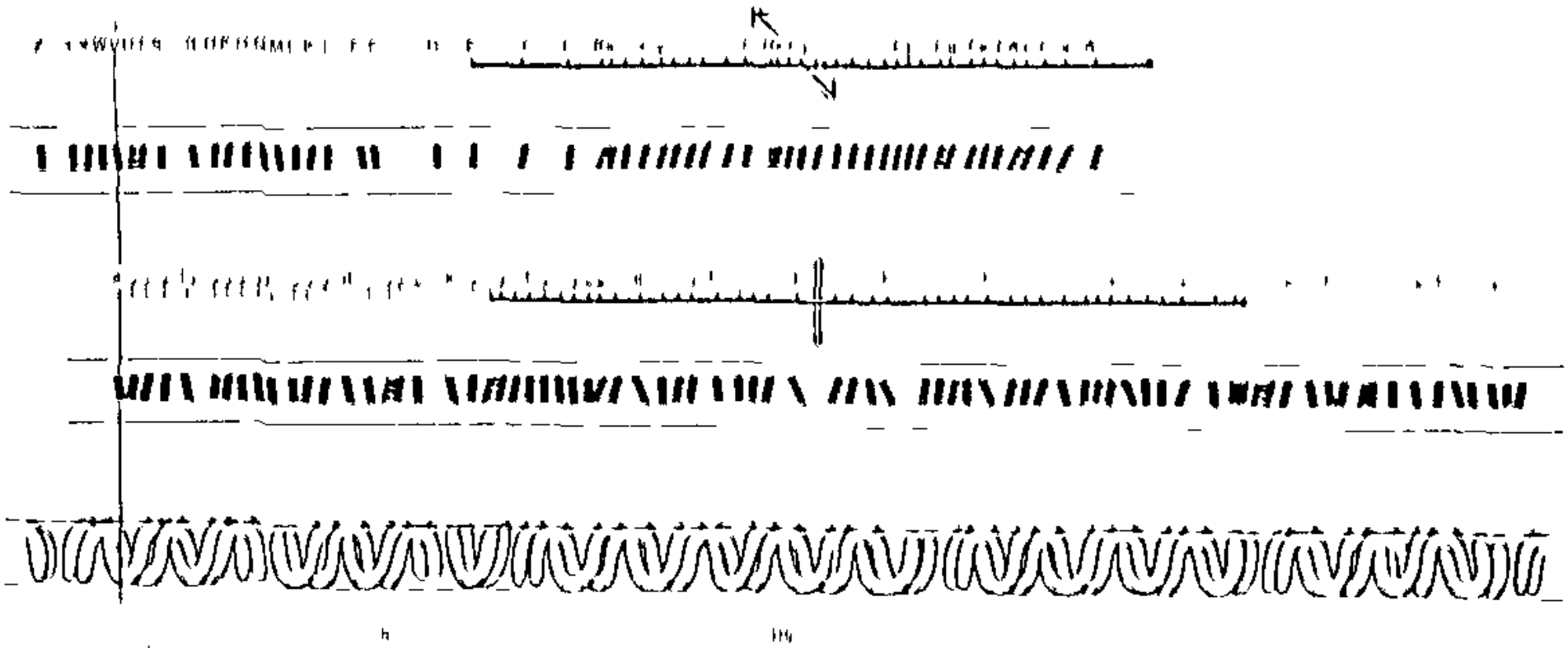


Fig. 8.12 Diagram showing a model of chromosome configuration in the cave cricket sperm as seen with polarized light. The chromosomes lie behind each other and show two orders of DNA supercoiling. (From Inoué and Sato, 1966, in Jerry Hayashi, Andrew G. Szent-Gyorgyi, Molecular Architecture in Cell Physiology, © 1966, p. 216. Reprinted by permission of Prentice-Hall, Inc., Englewood Cliffs, N.J.)

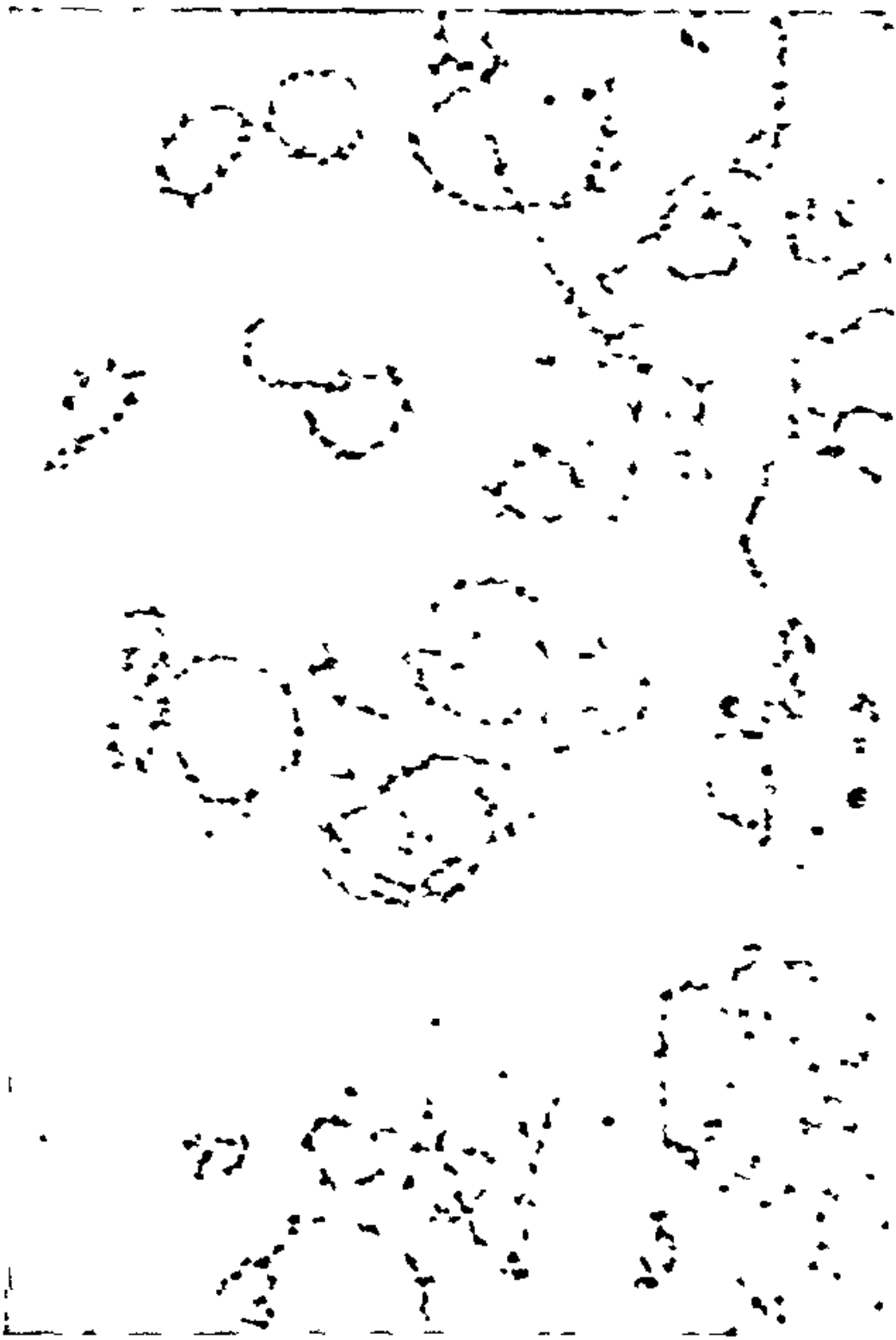


Fig. 8.14 Light microphotograph of circular DNA-containing ring nucleoli of amphibian oocytes (× 424) (From Miller 1966. Reprinted with permission of the National Cancer Institute, Bethesda, Maryland)

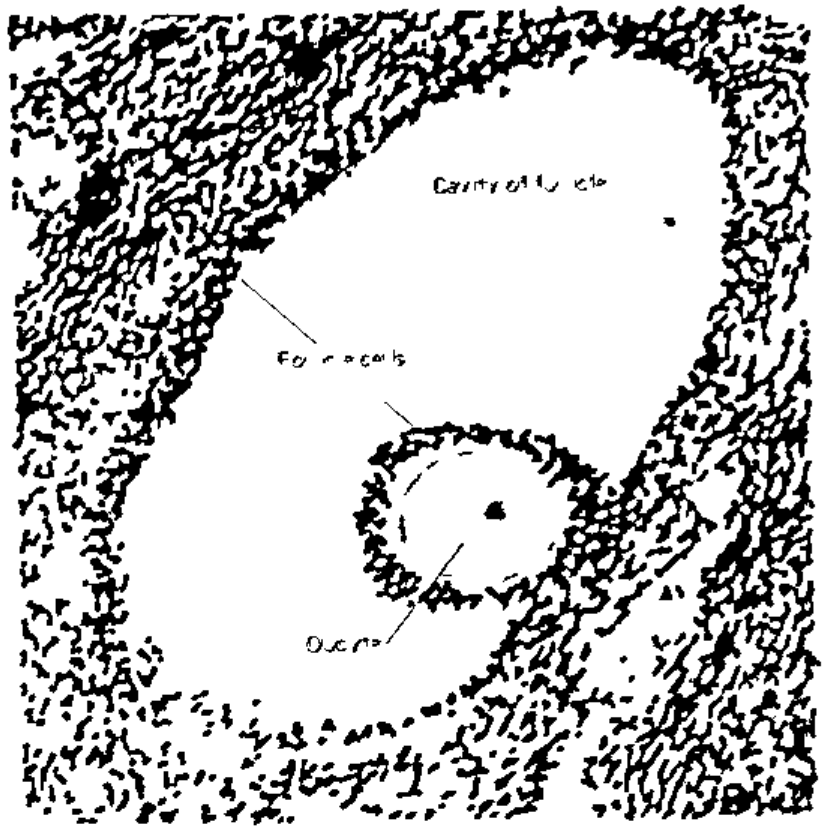


Fig 8 15 Nurse cells that surround the mammalian oocytes called *follicle cells* (From Balinsky 1970 Redrawn by permission of the W B Saunders Co Philadelphia)

The primary oocyte eventually resumes meiotic division At the end of meiosis I, two haploid cells are present, a large one, the secondary oocyte, and one small abortive cell, the so-called first polar body or polocyte (Fig 8 16) The polocyte remains attached to the oocyte Meiosis II also produces two cells of unequal size, a large functional egg called ootid or ovotid and a small abortive second polar body. During meiosis II the first polar body either disintegrates, remains undivided, or undergoes division to form a third polar body. In most species the three polocytes eventually disintegrate Figure 8 16 shows the actual process of the two meiotic divisions As the nuclear membrane breaks down at the end of prophase I, the chromosomes move from the center of the oocyte toward the periphery The division then actually appears as a bulging and pinching off of the small polar bodies from the large oocyte Two major purposes of this pinching off seem to be the elimination of half of the chromosomes by discarding them in the abortive primary polar body and a further growth of the oocyte that essentially receives all nutrient material because of unequal cell division This process completes the maturation of the oocyte into a mature egg

Fertilization or syngamy in animals is the process of sperm penetration (Fig 8 17) into the egg and the union of the paternal and maternal gametes resulting in zygote formation Thus, the homologous chromosomes that lost their partners during the process of male and female meiosis are now going to be matched again as homologous pairs This is the final step in the recombination of genes from different sources as mentioned before It makes possible the sharing of favorable genes

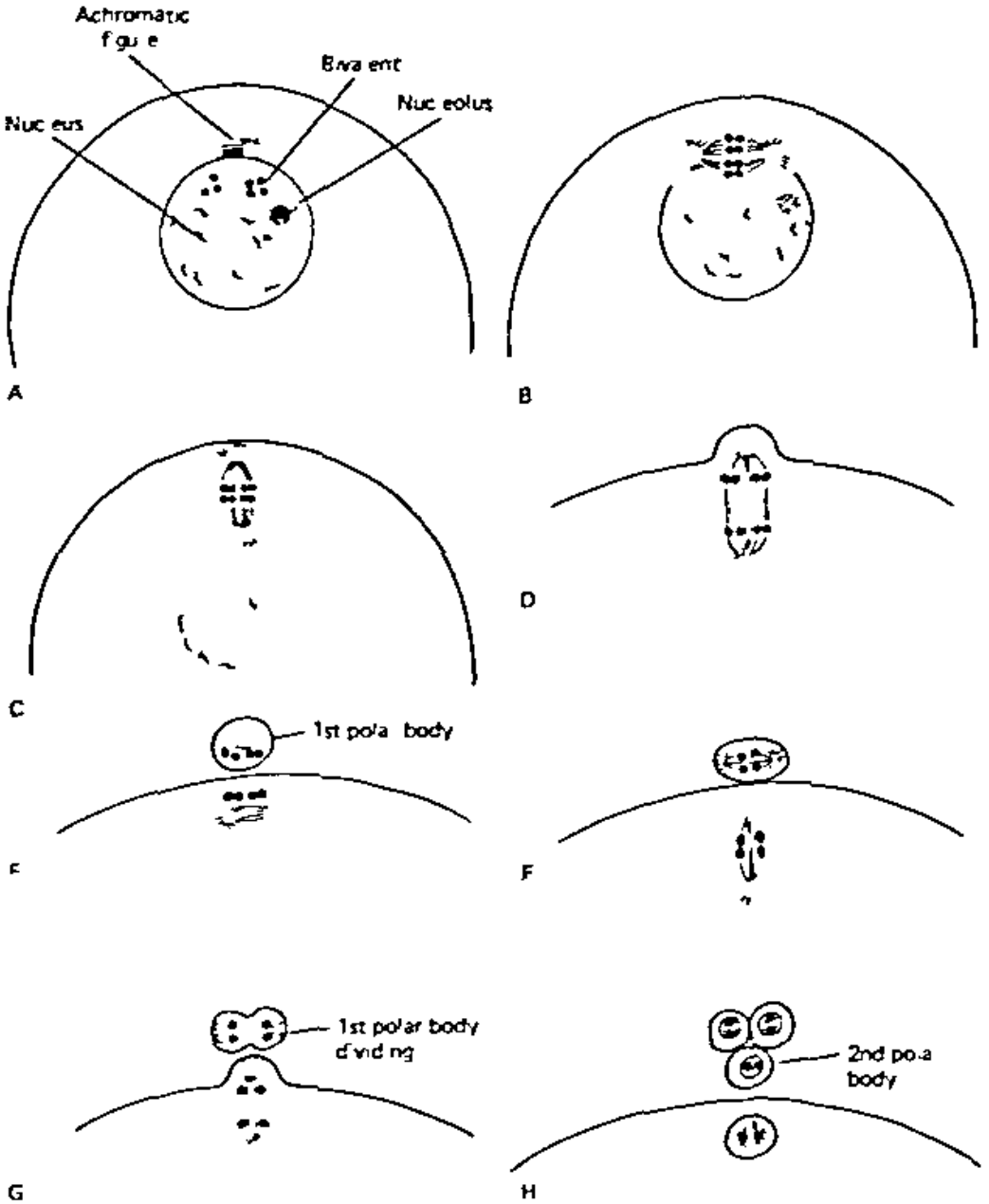


Fig 8 16 The two meiotic divis ons of the primary oocyte resulting in the maturation of the oocyte into a mature egg (From Bal nsky 1970 Redrawn by permission of the W B Saunders Co Ph ladelphia)

throughout a group of cross fertile individuals such as a species where before such genes were available only to one individual

Fertilization in animals usually starts before the completion of oogenesis Penetra tion of the sperm into the oocyte seems to be necessary for the maturation of the egg in a number of animal species In some mammals amphibians and insects the oocyte is still in the prophase I stage at the time of sperm entry The entrance of the sperm into the egg seems to activate the egg If the egg is not fertilized at all, it may eventually degenerate If it is fertilized, it goes into action If meiosis has not been completed then sperm entrance will bring it to completion

Fig 8 17 Electronm crograph of the process of sperm penetration in the sea urchin *Arbacia punctulata* (Courtesy of Dr Everett Anderson Department of Anatomy Harvard Medical School Boston Massachusetts)

The process of fertilization can be divided into three substages

1 The penetration of the oocyte membrane by the sperm
2 **plasmogamy** or the fusion of the cytoplasms of the two gametes
3 **karyogamy** or the fusion of the two pronuclei

The penetration of the oocyte membrane has been studied in detail by light and electron microscopy (Colwin and Colwin 1967) but it will not be discussed here in detail The mechanism of the sperm penetration seems to be spurred by a chemical in that the acrosome produces enzymes known as sperm lysine that dissolve the egg membrane locally (Tyler 1948 Colwin and Colwin 1961) Thus the acrosome seems to be a vital part of sperm penetration as it moves in front of the sperm with the nucleus centriole middle piece and tail trailing behind Often the tail breaks off as the sperm enters the egg cytoplasm Soon after entry the nucleus usually turns around 180 degrees so that the centriole is now in a forward position with the nucleus following behind Any other parts of the former sperm have now disconnected and disintegrate The nucleus and the centrosome of the sperm now both change their appearance The nucleus which was closely packed in the sperm becomes dispersed granular and enlarged (Longo and Anderson 1968) The centrosome forms an aster Both the sperm and egg nuclei become

similar in appearance and are now referred to as male and female pronuclei (Beneden 1875) In higher animals the pronuclei increase progressively in volume until they are about 20 times their original size (Austin 1969)

The female pronucleus also has to change position It is located at the periphery of the egg where it completes its meiotic division (Fig 8 16) As division is completed the female pronucleus also migrates toward the male pronucleus in preparation of the fusion of both This fusion generally takes place near the center of the egg Fusion of the two pronuclei varies from species to species In the sea urchin for instance fusion is complete at the onset In higher animals the two pronuclei suddenly diminish in volume and finally fade out altogether giving place to two chromosome groups These chromosome groups move together and form a single group *which represents the prophase of the first cleavage division* (Austin 1969) But such complete fusion does not occur in all animal groups In *Ascaris* some molluscs and in annelids the chromosomes of the male and female pronuclei attach to the zygote spindle and remain in two separate groups until completion of the first cleavage division Only then do the paternal and maternal chromosomes become enclosed by a common nuclear envelope In the fresh water crustacean *Cyclops* the paternal and maternal chromosomes remain in two separate groups until after the gastrulation stage of embryonic development The two groups actually can be observed forming bilobed nuclei Each group forms its own nuclear envelope But fusion of paternal and maternal chromosomes eventually occurs in all animal species

Part V
Variation in Chromosome Types

Chapter 9
Polyteny and Lampbrush Chromosomes

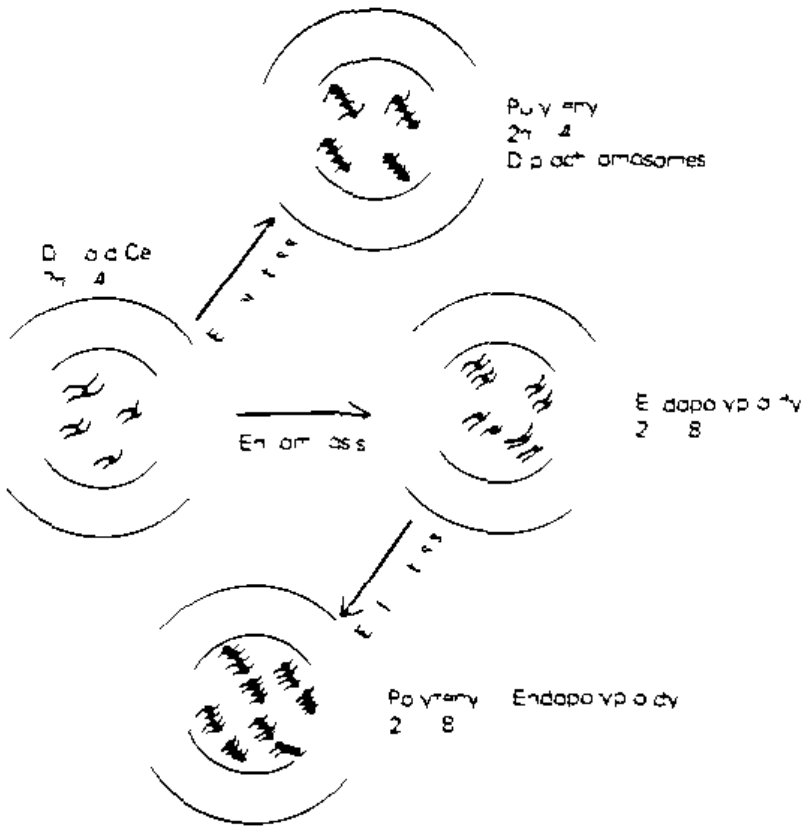
Beginning with this part a new phase of this book is introduced The preceding parts of the book dealt with the theme structure (Part II), function (Part III), and movement (Part IV) of the chromosomes The following parts of the book deal with the variations on the theme variations in chromosome types (Part V), variations of chromosome structure (Part VI) variation of chromosome number (Part VII), and variation of chromosome function and movement (Part VIII)

In the discussion of chromosome structure in Chapters 2 and 3, *normal* chromosomes usually seen during mitotic and meiotic cell divisions were described However in specialized tissues or in certain species or species groups, nonstandard or unusual chromosomes exist that serve as valuable tools for cytogenetic research The first group of such chromosomes to be discussed distinguish themselves by their unusual size These are the **polytene** and **lampbrush chromosomes.** These chromosomes reveal structural details that cannot be seen in ordinary somatic chromosomes

9.1 Polyteny vs Endopolyploidy

Polyteny (Koltzoff, 1934) is found in salivary gland nuclei, nurse cells and other larval tissues of dipterous flies and in intestinal cells of larval mosquitoes The phenomenon was discovered by Balbiani in 1881, but its cytogenetic significance and importance were not revealed until Kostoff (1930) Heitz and Bauer (1933), and Painter (1933, 1934) made their important contributions Heitz and Bauer's research was briefly mentioned in the historical treatise (Chapter 1)

Polyteny and **endopolyploidy** both are results of **endomitosis** (Geitler, 1937) Endomitosis is the process of chromosome duplication without karyokinesis and cytokinesis In endomitosis the DNA content of the cell is doubled or at least increased In polyteny the duplicated chromosomes do not separate into sister chromatids, while in endopolyploidy they separate, resulting in chromosome doubling Figure 9 1 demonstrates this difference If a diploid cell with 4 chromosomes ($2n=4$) goes through the process of endomitosis it can form a polytenic cell *in which the chromosome number does not change* ($2n=4$) *but the chromatid* number changes from 8 to 16, doubling the amount of DNA Each chromosome then has 4 chromatids instead of 2 White (1935) called such chromosomes


Polyteny
2n 4
Diploid chromosomes
Diploid Cell
2n 4
Endoreplication
Endomitosis
Endopolyploidy
2 8
Polyteny Endopolyploidy
2 8

Endopolyploidy is apparently a much more extensively occurring phenomenon than it was originally visualized Apart from the pathological occurrence of endomitosis in malignant tissues of mice (Levan and Hauschka, 1953), endopolyploidy is a normal process in plant and animal cells A review on the subject was published by Tschermak Woess (1963) In some diploid plants, for instance, the vascular tissues of the roots are polyploid (Jacobj, 1925) Such an increase in chromosome number is also characteristic of the mother cells from which latex tubes, vessel members, collenchema cells, and other specialized cells such as idioblast cells arise (Tschermak Woess and Hasitschka, 1954) Müntzing (1961) reports endopolyploidy in the roots of spinach Wipf and Cooper (1938) found that in legumes like red clover, common vetch and garden peas, the root nodules that are involved in nitrogen fixation have plant cells with twice the chromosome number observed in the rest of the plant In the vetch in addition to cells with the normal chromosome number $2n = 12$, cells with $2n = 24$, 48, and even 96 chromosomes have been reported Endopolyploidy in animals is best known in insects Geitler (1937, 1939, 1941) showed that in the salivary glands of the water insect *Gerris lateralis* ($2n = 21$, XO type), many cells were 512-ploid, some 1,024-ploid, or even 2,048-ploid

9 2 Morphological Characteristics of Polytene Chromosomes

The **polytene chromosomes** have been most thoroughly studied in the salivary glands of *Drosophila* Their discovery has been briefly mentioned in Chapter 1 Bridges was one of the most diligent researchers in mapping these **giant chromosomes** The length of these chromosomes in the late larval stage of *Drosophila* is about 100 times the length of somatic chromosomes They are believed to be in the interphase stage and their enormous length could be explained by molecular unfolding According to the **polyteny hypothesis** of Bauer (1935), these chromosomes originate from repeated endomitotic cycles forming bundles of numerous chromonemata (chromatids) that are held together by **somatic pairing** and consequently they are represented in the haploid number Each so-called **polytene chromosome** really represents a very close union of two homologous chromosomes that simulate the appearance of only one single chromosome

In a typical salivary gland cell of *Drosophila melanogaster* the four chromosomes are all united in the chromocenter by their centromere regions (Fig 9 2) This gives the cell the appearance of five long and one short strand radiating out of the chromocenter The strands represent the arm of the telocentric X chromosome, two arms of chromosome 2 (2R, 2L), two arms of chromosome 3 (3R, 3L), and the tiny arm of the small telocentric chromosome 4 Along these arms appear the distinct darkly stained **bands** that divide the chromosomes into band and **interband** regions These bands are of different size and staining capacity, and some of them appear as **doublets.** According to Lewis (1945), doublets represent **one-band-tandem-repeats.** The bands are visualized as chromomeres that associate closely at the same level of the interphase chromonemata Each band really is a disk that


cldox
ADH
ODH
3L
2L
2P
3P
SCALE
50 μm

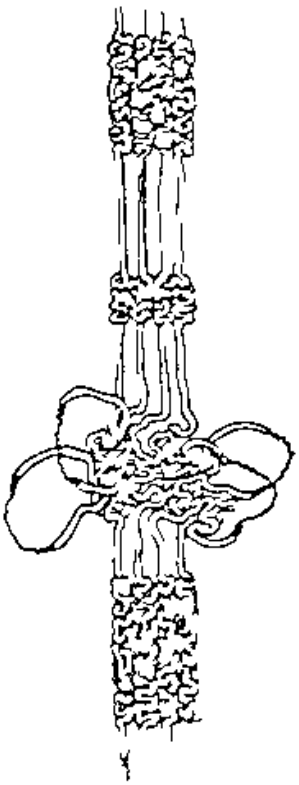


Fig 9 3 DuPraw s model of a polytene chromosome consist ing of multiple side by side strands each corresponding to an unfolded metaphase unit chromatid Concentrated bands of DNA arise by tight folding in all the unit chromatids at spe cific s tes At some sites DNA may unpack for RNA synthesis leading to the formation of a puff (From DuPraw and Rae 1966 Redrawn by permission of Macmillan Journals LTD., London)

Polytene chromosomes have also been reported in plants They were detected in certain cell types of the embryo by Tschermak Woess (1956) Hasitschka (1956) and Nagl (1962 1965 1969a) Nagl (1967 1969b 1970) and Avanzi et al (1970) also found polytene chromosomes in the suspensor cells of two species of *Phaseolus* and they studied the structure and function of these chromosomes in detail

9 3 Puffing

Since the early discovery of the salivary gland chromosomes by Balbiani it has been known that they may have a number of small to large swellings on them that were later called Balbiani rings and puffs Balbiani rings are restricted to the Chironomidae while puffs occur in all Diptera Balbiani rings are extremely large puffs There are also some structural differences between Balbiani rings and puffs Puffing seems to throw light on the metabolic function of the giant interphase chromosomes that seemed to puzzle cytologists for a long time The first one to observe this phenomenon was Bridges (1935) Puffing involves an unfolding of DNA in the band regions (Fig 9 5) Such bands originally have very distinct outlines but with progressive puffing they become more diffuse until they disappear completely This process is reversed as puffs disappear Puffing also can spread to other adjacent bands (Pelling 1966) Apparently there is a difference between RNA puffs and DNA puffs Both kinds of puffs are unusually active in RNA synthesis but in the DNA puffs additional DNA is produced during the time of puffing DNA puffs

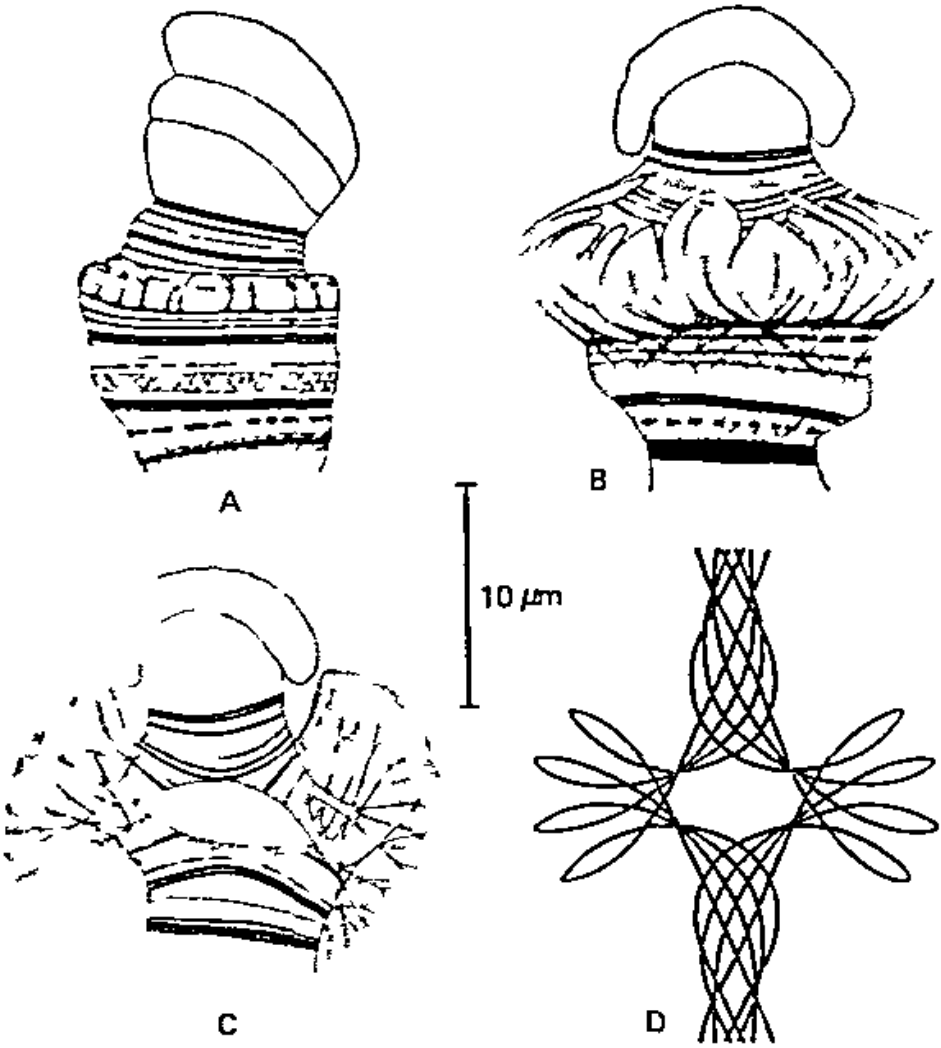


Fig 9 5 Different degrees (*A* to *C*) of puffing of the Balbiani ring in the salivary gland chromosome IV of *Chironumus tentans* The interpretation of the course of the chromosomal fibers in the region of the Balbiani ring is given in *D* (After Beerman, 1952, from Clever, 1964)

(1971) **general chromosome model** that suggests that the fibrous coding DNA is in the interbands and that the globular DNA in the bands is control DNA (see Section 3 4 4)

9.4 Super Chromosomes

The extent of polyteny can be increased by infection with microsporidian protozoan parasites (particularly of the genus *Thelophania*) Such parasites cause the salivary gland cells to grow very large and to produce **super chromosomes.** Such chromosomes have been studied by Diaz and Pavan (1965), Pavan and Basile (1966), Pavan (1967), Pavan and DaCunha (1968), and Diaz et al (1969) When super chromosomes are stained, they are visible to the naked eye They may have from

Fig 9 6 The four (X A B C) polytene super chromosomes of an infected salivary gland cell of *Rhynchosciara angelae* Scale 50μm (Courtesy of Dr C Pavan University of Campinas Sao Paulo Brasil Reprinted by permission of the C V Mosby Company Saint Louis)

250 000 to 1,000,000 chromonemata Roberts et al (1967) estimated that nuclei infected by parasites may have 2, 4, 8, 16 or 32 times the DNA content of normal salivary gland nuclei This indicates that up to five extra cycles of replication may have occurred Puffing was inhibited in larvae of *Rhynchosciara* that were infected by parasites In Fig 9 6 super chromosomes of an infected salivary gland cell of *Rhynchosciara angelae* can be seen

9.5 Somatic Synapsis

Synapsis is generally considered to be a meiotic process However, as we have seen in this chapter, it is also occurring in other types of cells Depending on the closeness of the pairing association of the homologous chromosomes in such pairing configurations, the phenomenon is either called **somatic pairing** or **somatic synapsis.** Somatic pairing has been known for a long time in plants (Strasburger, 1904, 1905, Sykes 1908 Digby, 1910 Nemec, 1910) and animals (Montgomery, 1906, Stevens 1908) The most striking type of somatic pairing exists in the Diptera where the homologous chromosomes lie next to one another and closely parallel throughout interphase Somatic pairing is less close at metaphase The most intimate pairing is observed in the salivary gland chromosomes, where it is usually associated with synapsis There is, however, an important difference between meiotic and somatic synapsis Meiotic synapsis is always two-by-two If more than two homologous chromosomes are present during meiotic synapsis (in trisomics or triploids Chapters 15 and 16), only two-by-two pairing occurs at any given section of the chromosomes *In the somatic synapsis of salivary gland chromosomes, pairing* occurs three-by three in triploids (Fig 9 7)

9.6 Lampbrush Chromosomes

As already mentioned in Chapters 7 and 8, the so-called **lampbrush chromosomes** are another type of giant chromosomes that occur in the diplotene stage of primary oocyte nuclei in vertebrates and invertebrates and also in the Y chromosome of *Drosophila* spermatocytes Lampbrush chromosomes can be even larger than the polytene giant chromosomes of the Diptera, but their diameter is much less The longest such chromosomes of about 1 mm have been found in urodele amphibia (Rieger et al , 1976) These chromosomes are characterized by a typical diplotene bivalent appearance showing chiasmata and from the darkly stained chromosomes *thousands of thin chromatin loops are extending laterally at rectangles (Fig 9 8)*

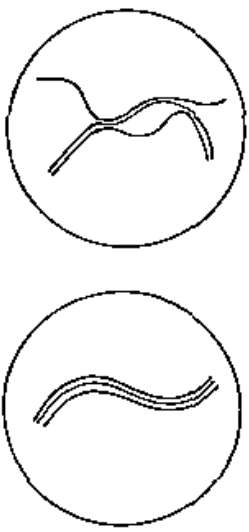


Fig 9 7 Comparison of chromosome pairing in meiotic and somatic synapsis

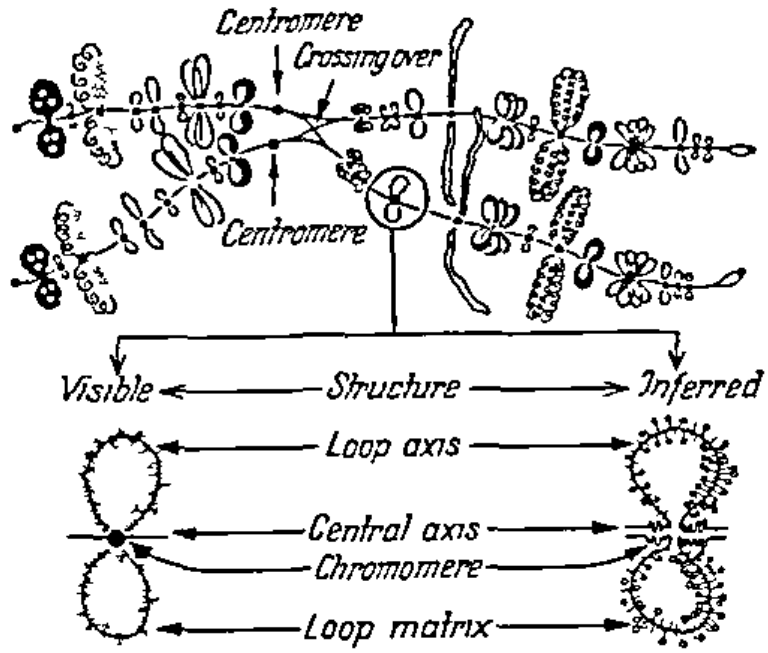


Fig 9 8 Diagram showing typical appearance of a bivalent consisting of two lampbrush chromosomes (From Lewis and John 1963)

The first lampbrush chromosomes were discovered by Rückert (1882) in shark. Almost all of the recent research has been with the urodeles particularly of the genus *Triturus* The most improved techniques in studying lampbrush chromosomes were applied by Gall and Callan (1962) A phase-contrast photograph of a portion of an isolated lampbrush chromosome is shown in Fig 9 9

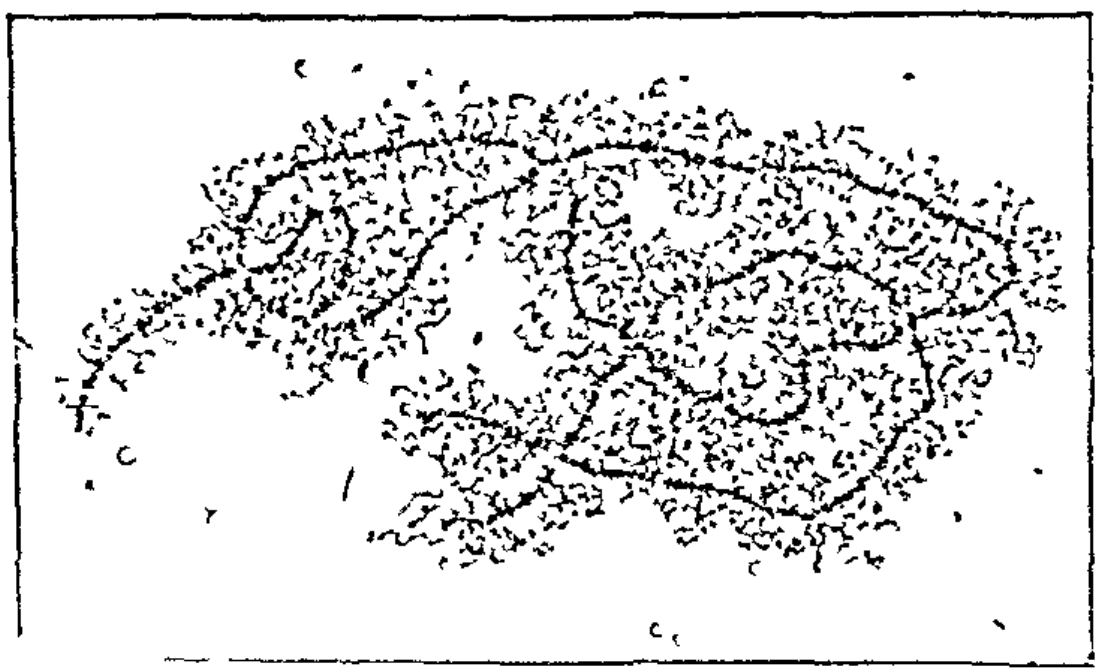


Fig 9 9 Phase contrast photograph of a portion of a pair of lampbrush chromosomes isolated from an oocyte of the newt *Triturus viredescens* (× 337) (From Gall 1966)

Beermann (1952) first realized that the lampbrush chromosome loops are similar in function to the puffs of the polytene chromosomes. But certain major differences are apparent between the puffs and the lampbrush loops. A puff consists of thousands of identical DNA loops, each arising from a chromomere at the puff producing locus. While in the lampbrush chromosome, all or almost all chromomeres have formed loops, in the polytene puff only a few unfold at any one stage of development or in any particular tissue (White 1973)

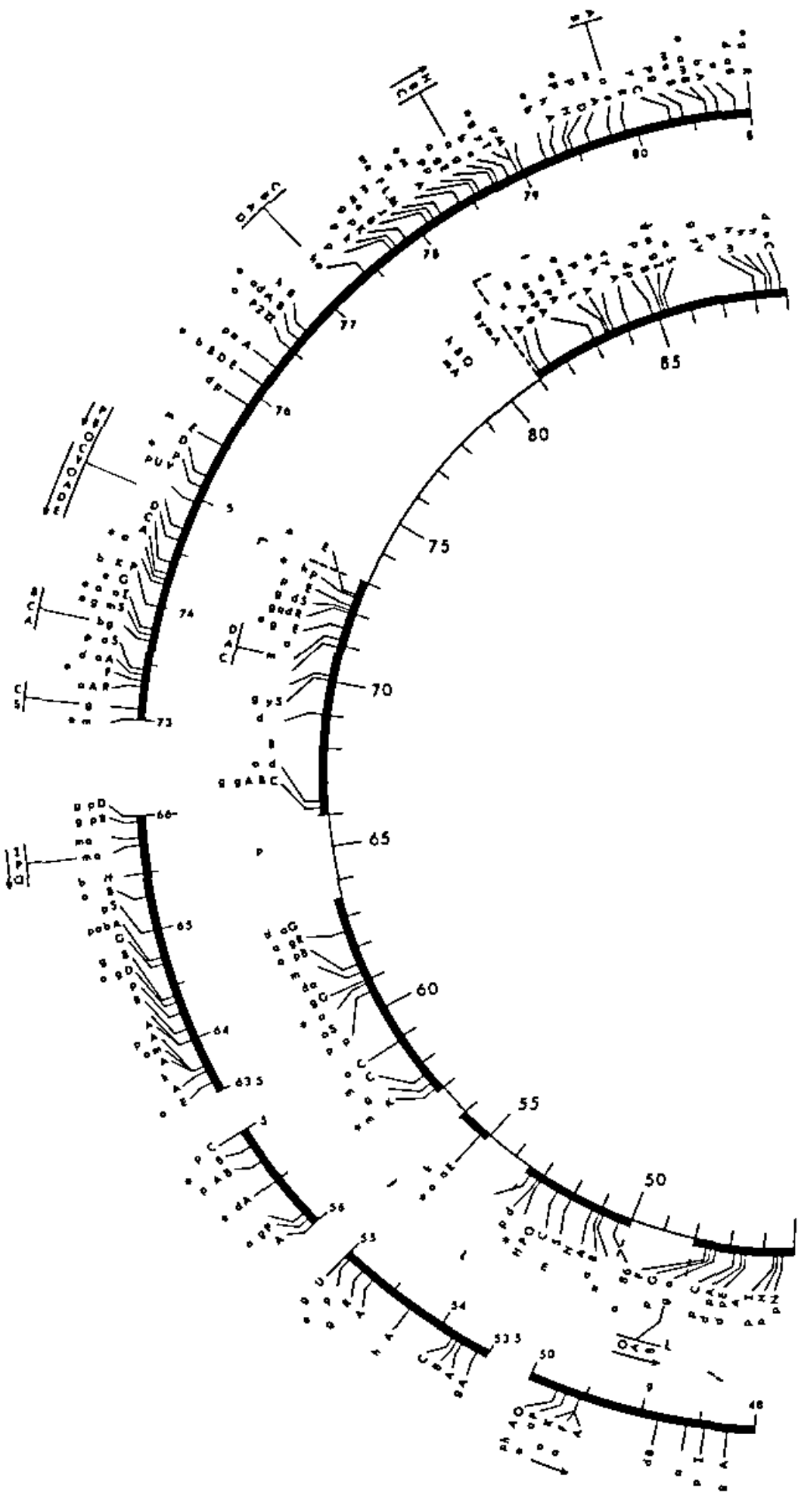
DuPraw (1970) mentions that each pair of loops along a giant lampbrush chromosome has its own specific morphology and often one side of the loop is thicker than the other as if more RNA had accumulated there This suggests that the loops are units of genetic activity Apparently along each loop mRNA transcription occurs

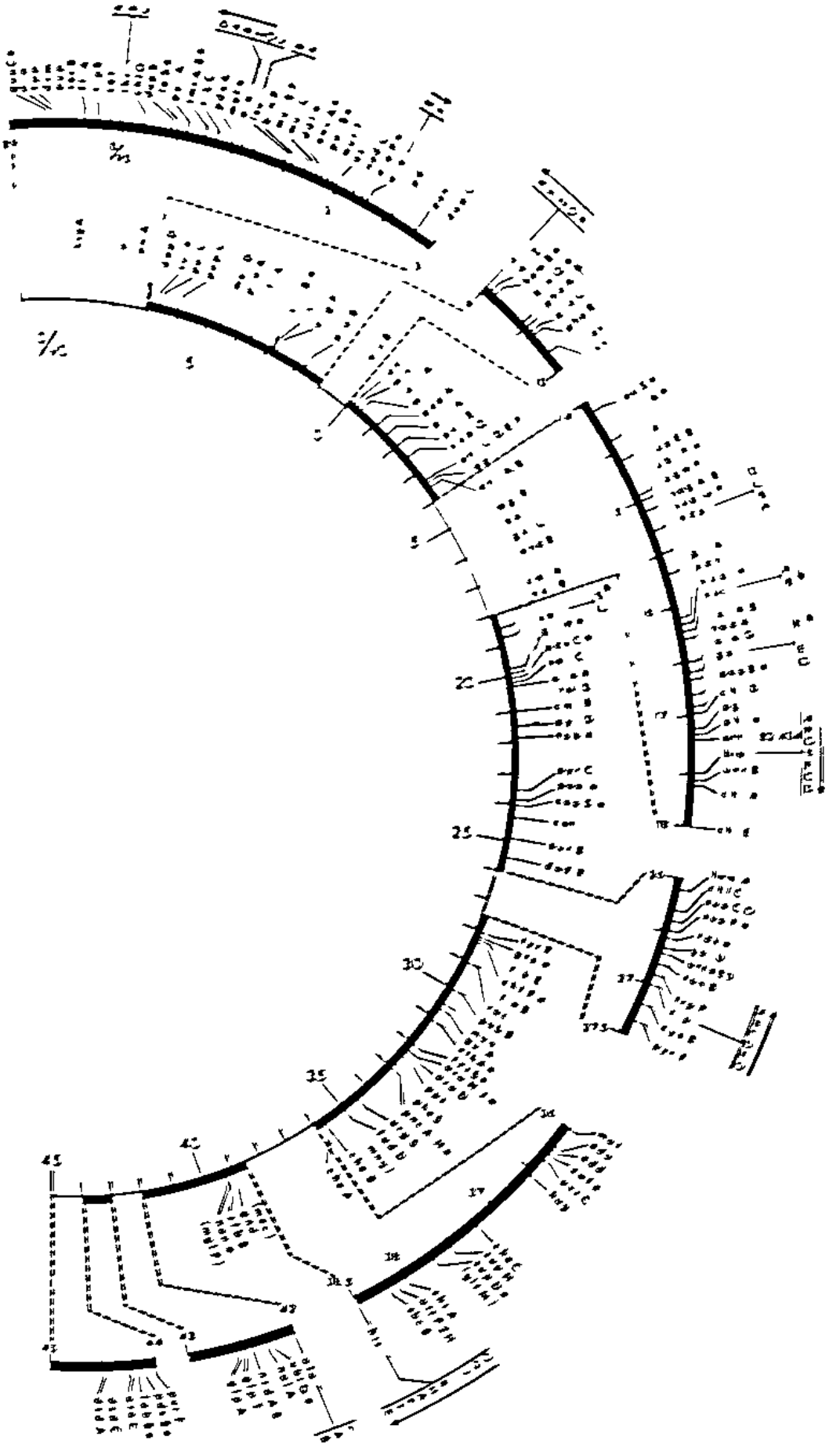
Chapter 10
Ring-Chromosomes, Telocentric Chromosomes, Isochromosomes, and B Chromosomes

This chapter is a continuation of the discussion of unusual chromosome types As mentioned before the term "unusual' in this connection is a relative term We very often have a certain concept of things and whatever deviates from this concept we call "unusual" Because **ring-chromosomes, telocentric chromosomes, isochromosomes, or B chromosomes** *differ from the majority of chromosomes in* humans, animals and plants, they are considered unusual But apparently, in some instances, such chromosomes fulfill a need that cannot be met by any other chromosome type There is no obvious connection between these four chromosome types except that they all deviate in some way or another from the prototype as described in Chapters 2 and 3

10.1 Ring-Chromosomes

Standard chromosomes of higher organisms (**eukaryotes**) usually have two ends and do not form a continuous ring However, the chromosomes of lower organisms such as **prokaryotes** (e g , bacteria like *Escherichia coli* and some viruses) normally have ring-shaped chromosomes Often such chromosomes are referred to as **genophores** (Ris, 1961) in order to emphasize the difference in structure A linkage map of *E coli* is shown in Fig *10 1* It demonstrates the circular structure of the bacterial genophore Such genophores are more than 1 mm in length and consist *of a single DNA molecule that is tightly packed into a* **nucleoid** *(bacterial nucleus* without a nuclear envelope) of only 1 μm in length The DNA in such genophores is considered to be naked or pure, seemingly lacking any kind of histones that are generally associated with DNA in the chromosomes of eukaryotes and carrying less protein The diameter of a genophore of *E coli* is reported to be 4 nm, which is twice the diameter of a Watson-Crick double helix (Miller et al , 1970) A possible **unidirectional model** for DNA replication of circular chromosomes has been developed by Cairns (1963) This model is shown in Fig 10 2 Replication starts at a fixed point and always proceeds in the same direction Because the complementary strands of the DNA molecule are twisted in a double helix, the circular DNA must rotate around its own axis as the old and new strands separate Another more recent model indicates that DNA replication of the circular *E coli* chromo-





Trotter, 1972. Reprinted with permission of the American Society of Microbiology, Washington, DC)

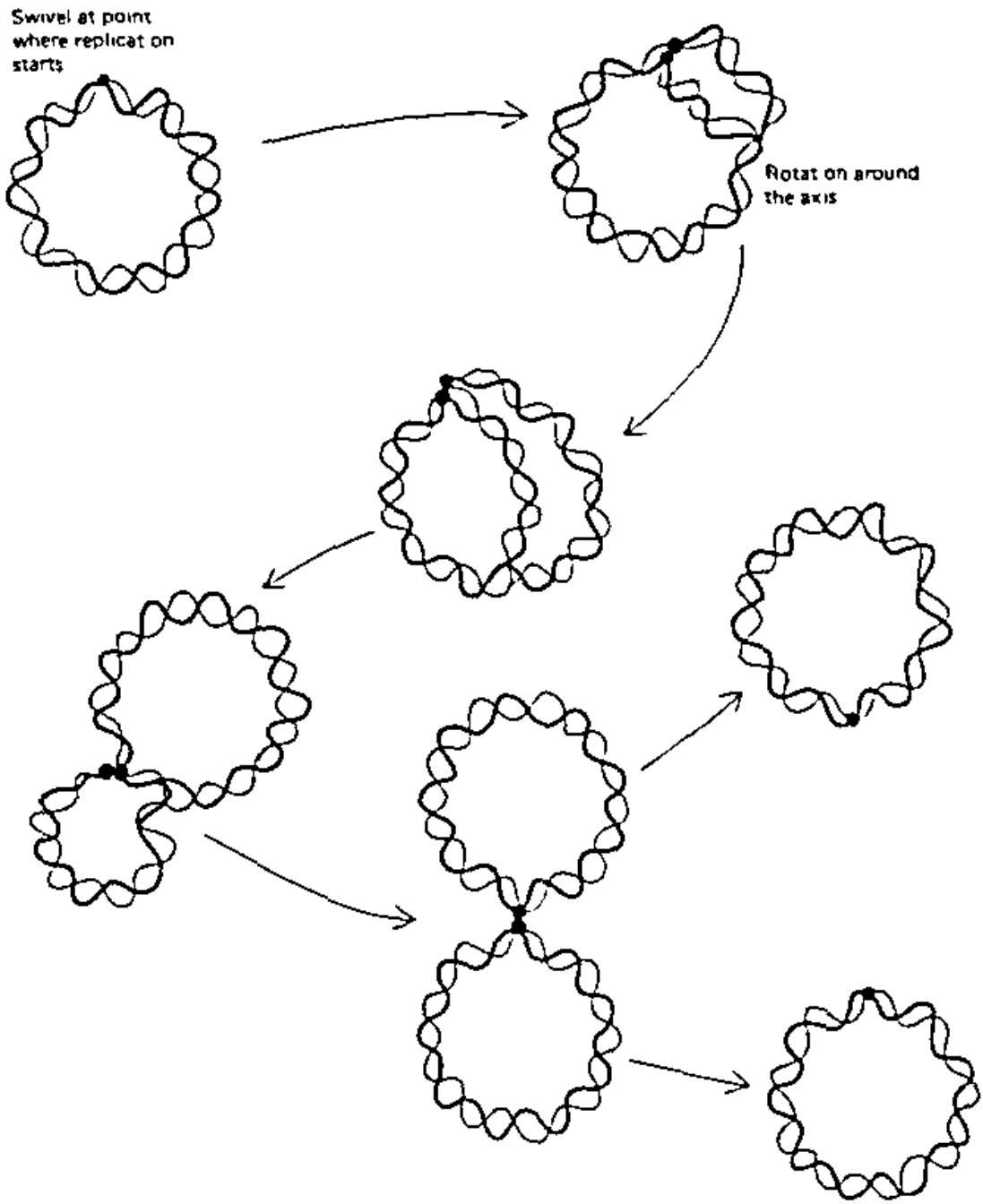


Fig 10.2 Diagram of a possible model for circular DNA replication (From Cairns 1963 Redrawn by permission of the Cold Spring Harbor Biological Laboratory, Cold Spring Harbor New York)

some proceeds in a ***bidirectional fashion*** from a fixed point (Masters and Broda, 1971)

Apart from such normally occurring ring-chromosomes in prokaryotes, such chromosomes frequently form in eukaryotes as a result of structural chromosome changes Chromosomes in higher organisms are not naturally ring-shaped Ring chromosomes have been detected in humans, *Drosophila* and certain plant species They were most thoroughly studied in maize by McClintock (1938b, 1941a, 1941b, 1944) In maize, ring-chromosomes are likely to form dicentric double sized rings

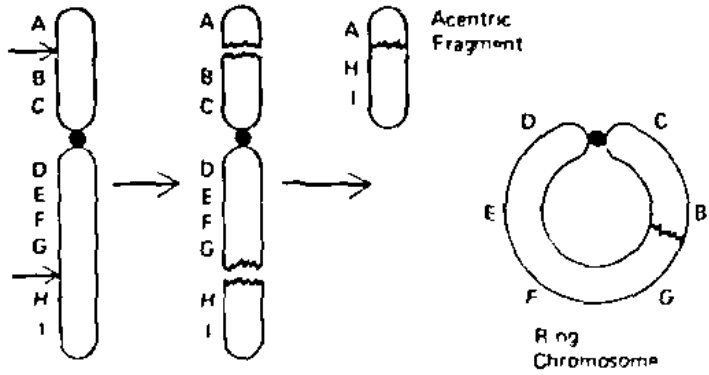


Fig 10 3 Diagrammatic presentation of the formation of a ring chromosome

that break at anaphase This very often leads to instability of the rings The details of this behavior will be discussed in Chapter 12 Normal chromosomes do not form rings because they are believed to have telomeres on each end (Chapter 3) Telomeres seem to prevent the union of chromosome arms into ring formation A chromosome can form a ring-chromosome by fusion of the raw ends (Fig 10 3) only if it has two terminal deletions (Chapter 12) producing a centric segment with two raw ends and two acentric fragments As seen in the illustration the ring chromosome (BC DEFG) inherits the centromere and the terminally deleted material can unite into an acentric fragment (AHI) that eventually gets lost from the nucleus A ring chromosome lacks the genetic information that was carried by the terminally deleted fragments After the occurrence of such a deletion an organism or tissue will be heterozygous for the deletion having a normal standard chromosome (ABC DEFGHI) and a deleted ring chromosome (BC DEFG) Ring chromosomes generally are meiotically unstable During mitosis they can produce two daughter rings of equal size that are regularly distributed to the daughter cells Such ring-chromosomes can be somatically stable

Ring-chromosomes have been observed in several human syndromes According to Borgoankar (1975), they involve all human chromosomes except 2, 10, 11, and 12 Since 1975 a ring chromosome 2 was found in a newborn (Vigfusson, unpublished) A karyotype of the ring-chromosome 2 individual is shown in Fig 10 4 Figure 10 5 shows an enlarged photograph of homologous chromosomes 2, one of them being the ring chromosome

Eight children with heterozygous ring chromosome conditions in the E group (Er) (see Fig 2 3) had generalized mental and developmental retardation and a variety of congenital malformations (De Grouchy et al 1968 Hamerton 1971b) Such clinical features could be expected since the ring chromosome necessarily involves two terminal deletions In these instances the ring chromosomes were unstable Another eleven patients were observed with 2n = 46 chromosomes and with a ring chromosome replacing one of the standard chromosomes in the D group (Lejeune et al , 1968a, Hamerton, 1971b) The majority of these had developmental and mental retardation and variable congenital malformations It could not be established if any of these ring chromosomes involved the same D chromosome In six of the eleven D ring chromosome (Dr) patients, the rings seemed to be relatively

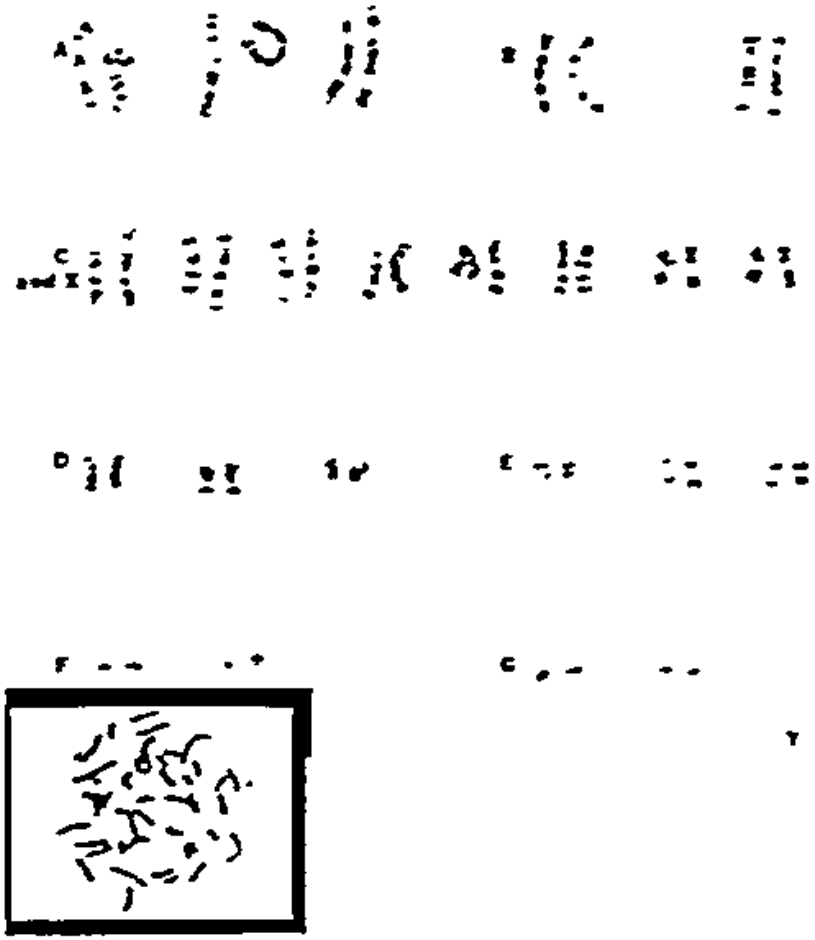


Fig 10.4 Human G-banding karyotype of a newborn with a ring chromosome 2. Insert. Original cell. (Courtesy of Dr N V Vigfusson, Department of Biology, Eastern Washington University, Cheney Washington)

stable. Consequently, the D rings seem to be more stable than the E rings. C ring stability was studied by Shaw and Kreeth (1966) over about 70 cell generations. The proportion of the cells containing ring-chromosomes diminished with time and they disappeared completely in two cell lines. A case of ring-chromosome mixoploidy was observed in a female infant with apnoeic spells (partial suspension

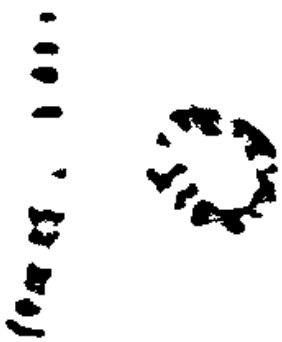


Fig 10.5 Normal and ring-chromosome 2 of same patient as in Fig. 10.4 (Courtesy of Dr N V Vigfusson)

of breath), abnoral ears, and hypoplastic (below normal size) nails Mixoploids (Nemec, 1910) are chimeras in which the cells vary according to their chromosome numbers In this case the blood cultures revealed two different cell lines one normal (46, XX) and one trisomic (47, XX r+) The trisomic cell line had a ring chromosome in addition to the normal cell complement The ring-chromosome varied in size from a G group to a D group chromosome (see Fig 2 3) The proportion of cells containing the ring-chromosome diminished from 20% in early blood cultures to 8% in blood cultures taken at the age of 13 months (Hamerton 1971b) A probable case of an A ring-chromosome (Ar) was recorded by Cooke and Gordon (1965)

Other ring-chromosome syndromes in humans causing congenital malformations have been recorded by Smith White et al (1963) Aula et al (1967) and Gripenberg (1967) In some of these the X chromosome formed the ring

Ising and Levan (1957) observed one or more ring-chromosomes in some cells of lung and stomach carcinoma with chromosome numbers between 2n=70 to 2n=80 Ring-chromosomes form various kinds of syndromes in men that are expressed more or less severely according to the size of the deletions involved If only a small amount of genetic information is missing the patient may be affected little or not at all

Reasonably stable stocks of *Drosophila* having a ring shaped X chromosome have been maintained in the laboratory (Morgan, 1933, Schultz and Catcheside, 1937 Swanson, 1957) Brown et al (1962) discovered that such stocks showed mosaicism (mixoploidy) as a result of somatic crossing over and because of ring elimination A ring Y-chromosome in *Drosophila hydei* was described by Beck et al , 1979

10.2 Telocentric Chromosomes

Telocentric chromosomes (Darlington, 1939a) or **telocentrics** are those that have a terminal centromere They are generally not considered to exist in nature but are formed by centromere misdivision If at mitosis the centromere divides transversely instead of longitudinally (Fig 10 6), the result is two telocentric chromosomes each of which inherits a part of the original centromere Since apparently *the entire centromere is required for normal centromere function, such telocentric* chromosomes are usually eliminated after a few cell divisions If, however, isochromosomes (Section 10 3) are formed from telocentrics, such chromosomes *seem to be maintained and stabilized Isochromosomes* can form if the telocentric arm reduplicates in interphase and the two chromatid arms do not become completely separated during the next mitotic interphase but stay united at the half-centromere The telocentric then becomes a metacentric isochromosome (Fig 10 6) Such chromosomes have two identical arms that are, genetically speaking, homologous

There is some reason for the fact that a centromere is not completely functioning if it does not have chromosome arms on both sides It is possible that the centromeric properties reside in the chromomeres flanking the unstainable centromere on either side (White, 1973)

Telocentrics originating from centromere misdivision have been reported in maize,

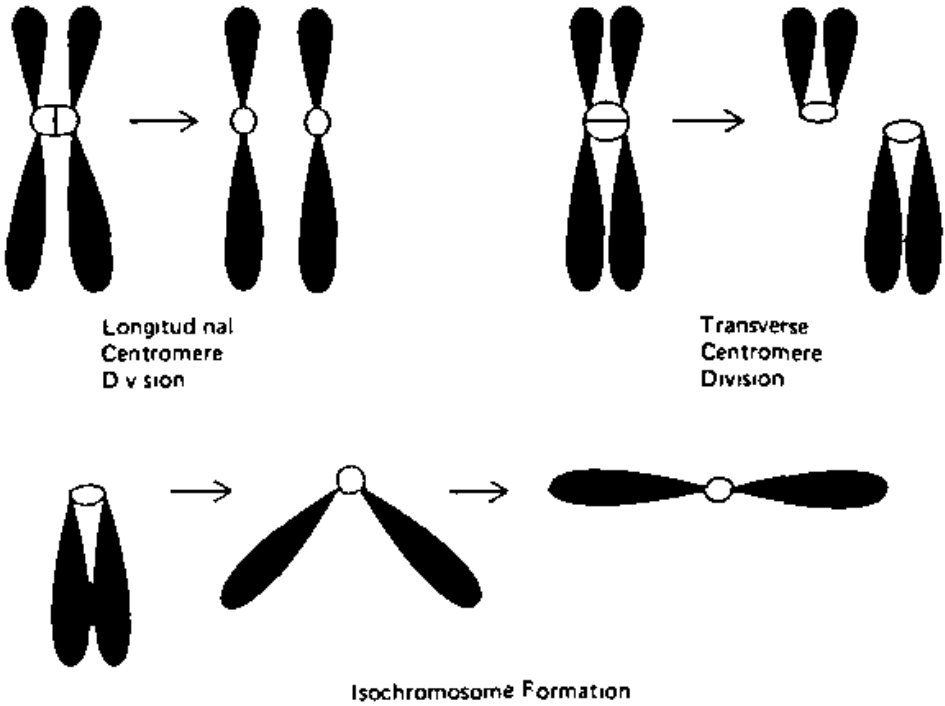


Fig 10 6 Diagrammatic representation of isochromosome formation

wheat, cotton, and tomato Maize telocentrics were reported by McClintock (1932) and Rhoades (1936, 1938, 1940) They are mitotically unstable The telocentrics of wheat (Sears, 1952a, 1952b, Morris and Sears, 1967, Sears, 1969) are not as unstable as those of maize and can be called semipermanent Brown (1972) suspects that the centromere fractions in wheat telocentrics are more than just half-centromeres Sears (1962, 1966, 1969) reports the successful use of wheat telocentrics in gene mapping Telocentrics are now available for most of the 42 chromosome arms in wheat Most of them are maintained as ditelosomic lines A ditelosomic line in wheat ($2n=42$) is one that has two homologous telocentric chromosomes in addition to 20 normal chromosome pairs ($20^{II}+2^{t}$) The absence of a whole chromosome arm in a telocentric allows positioning of a gene in that arm as well as determining the distance of that gene from the centromere One advantage of mapping genes with telocentrics is the fact that most telocentrics are transmitted poorly through the pollen Endrizzi and Kohel (1966) mapped three chromosomes in cotton by the use of telocentrics Khush and Rick (1968c) have used telotrisomics to map genes in tomato A telotrisomic is a normal disomic with an extra telocentric chromosome in addition ($2n+t$) So-called "natural telocentrics" have been reported for Protozoae by Cleveland (1949), for Crustaceae by Melander (1950a, 1950b), for mouse by Tjio and Levan (1954), for cattle by Melander (Melander and Knutsen, 1953, Melander, 1959), for grasshoppers by John (John and Hewitt, 1966, 1968, John and Lewis, 1968), and for fish by McGregor (1970) Such observations may really involve **acrocentrics**, which are often mistaken for telocentrics Acrocentric chromosomes (White, 1945) are those in which the centromere is very close to one end of the chromosome In line with what was stated before, White (1957, 1973, White et al , 1967) thinks that all

naturally occurring chromosomes cannot be telocentrics but are acrocentrics He believes that there is always a minute second arm even if it cannot always be seen by conventional techniques

10.3 Isochromosomes

Isochromosomes (Darlington, 1940) are metacentric chromosomes with two homologous arms Such chromosomes really are a reverse duplication of the constitution ABC CBA (see Fig 10 7)

Their origin by centromere misdivision and reduplication of the telocentric fragments has been explained in Section 10 2 (Fig 10 6) At meiosis isochromosomes can act in three different ways (Sen, 1952, Elliott 1958)

1 internal pairing
2 fraternal pairing
3 normal pairing

In **internal pairing** the two arms of the isochromosome pair with each other in pachytene (**autosynapsis**) and after terminalization form a **ring univalent** at the end of first prophase in diakinesis (Fig 10 7A) In **fraternal pairing** one or both of the arms of the isochromosome pair with a homologous arm of another chromosome (Fig 10 7B), this can happen if the carrier is a secondary trisomic (Chapter 16) in which the isochromosome exists as an extra chromosome ($2n+1$) In **normal pairing** the isochromosome pairs with another one just like it (Fig 10 7C)

The attached X chromosome of *Drosophila* is a classic example of an isochromosome (Morgan, 1922) This chromosome consists of two normally acrocentric X chromosomes attached at their centromeric regions and possessing a single centromere The origin of this chromosome is not known This attached X causes a

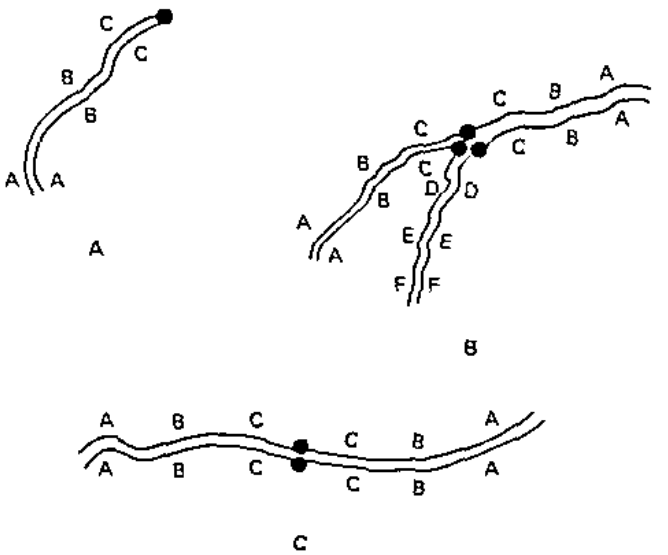


Fig 10 7A–C Different possible ways of meiotic chromosome pairing if an isochromosome is involved (*A*) Internal pairing (*B*) Fraternal pairing (*C*) Normal pairing

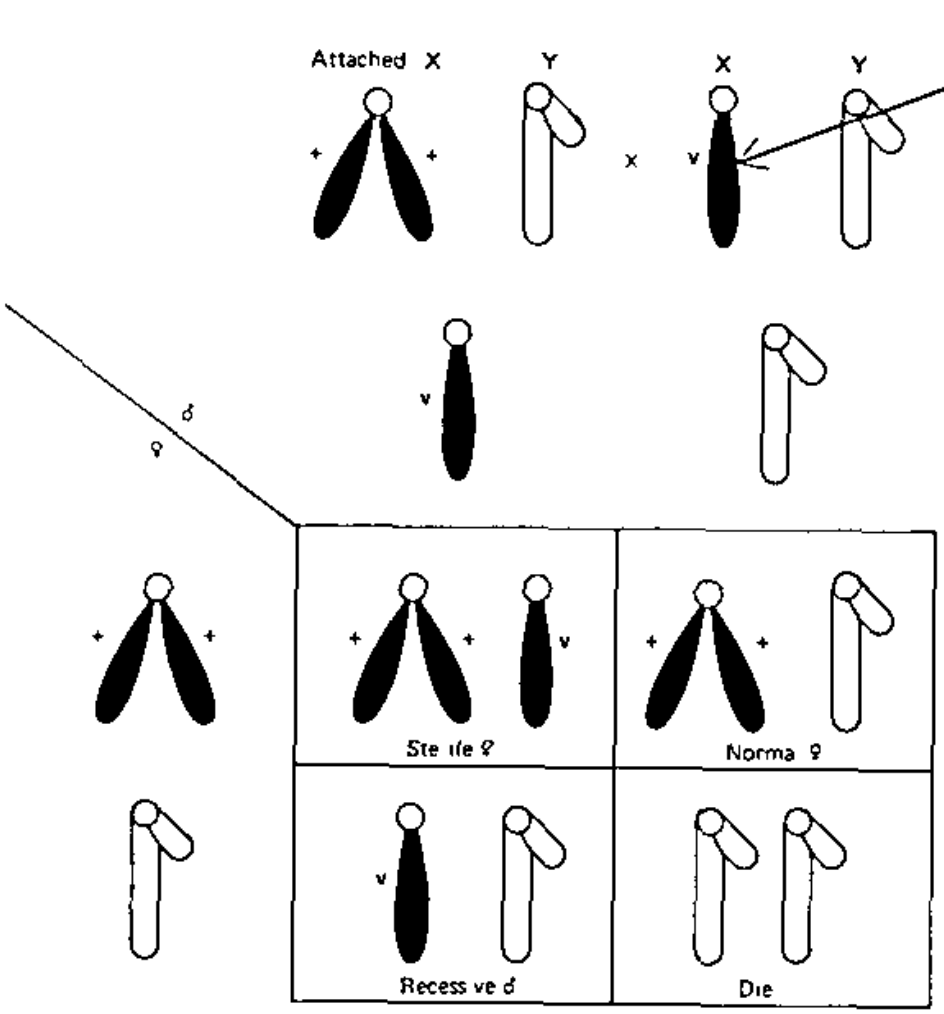


Fig 10 8 Diagram of noncrisscross inheritance as caused by attached X chromosome in *Drosophila melanogaster* showing transmission of *vermilion* eye color (*v*) (After Morgan 1922 Redrawn by permission of the Marine Biological Laboratory Woods Hole Massachusetts)

noncrisscross inheritance It can be used for detecting sex linked induced mutations (Fig 10 8) If XXY females with an attached X are mated with males in which a recessive mutation such as *vermilion* eye color (v) has occurred by x-ray treatment, then all the males of the next generation express this recessive character There apparently is a strong case for the occurrence of attached X chromosomes in humans The isochromosome in this case is formed by the two long arms of the X chromosome In an individual heterozygous for such an isochromosome (XX qi) the long arm of X is represented three times and the short arm only once (Fig 10 9) Such condition causes the phenotypic expression of an XO type or Turner syndrome (Chapter 15) Such **isochromosome Turner syndromes** can be diagnosed by unusually large Barr bodies that are formed by the iso-X (XX) (Brown, 1972) Most humans with an isochromosome are mixoploids (45, X/46, XX qi) (Hamerton, 1971a) The isochromosome is shut off and the functioning X behaves like an XO Turner syndrome

Other isochromosomes in humans involve the Y chromosome, the D group, and the

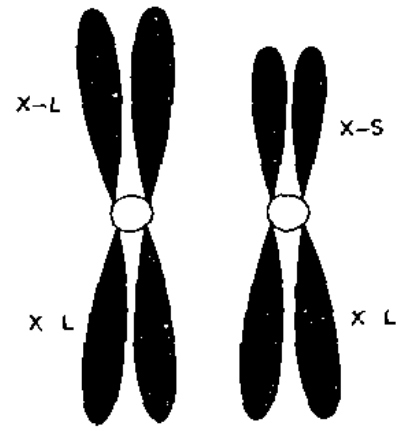


Fig 10 9 Diagrammatic representation of the heterozygous X isochromosome condition (XX qi) in humans

G group Jacobs and Ross (1966) found two females with one X chromosome and a possible isochromosome formed by two long arms of the Y (46, XY qi) The clinical characteristics of these individuals were ovarian dysgenesis (failure of menstruation), streak gonads, and primary amenorrhea (defect of the ovary) The fact that both cases expressed femaleness may indicate that the male determining factors of the Y are located on the short arm Another kind of isochromosome has been presumed for the long arm of a D group chromosome by Therman et al (1963) and by Giannelli (1965a) Therman et al found mixoploidy of two cell lines, one having a short arm deficiency in a D group chromosome, the other having a D group isochromosome (46, XX, Dp-/46, XX Dqi) They presumed that the Dqi line arose from the Dp- line during early cleavage Hamerton (1962) and Polani et al (1965) found an isochromosome that they believed involved the long arm of a G group chromosome Hsu (1969) found isochromosome heterozygosity in six out of seven investigated rats of the species *Sigmodon minimus* from New Mexico The isochromosome consisted of the fusion of the long arms of the two homologues of an acrocentric chromosome

Isochromosomes have been used to test the effect of colchicine on chiasma frequency (Driscoll and Darvey, 1970) Colchicine was applied to wheat after the last premeiotic mitosis until metaphase I with the result that chiasma frequency was reduced to about 50% of the normal level except in an isochromosome This seems to prove that the homologous regions involved in crossing over are subject to some physical forces that prearrange proximity of homologous segments prior to synapsis The two homologous portions of the isochromosome were held together by the centromere and were not affected by the colchicine

Also mentioned here are the **pseudoisochromosomes** that were obtained by x-radiation (Caldecott and Smith, 1952) These chromosomes are similar in their genetic constitution to isochromosomes in that the ends of their chromosome arms are homologous, but the chromosome segments next to the centromere (**interstitial chromosome segments**) are nonhomologous Pseudoisochromosomes are the result of reciprocal translocation between end segments of opposite arms of chromosomes of the same homologous pair (Fig 10 10) Internal pairing at meiosis of such chromosomes is like that shown for isochromosomes

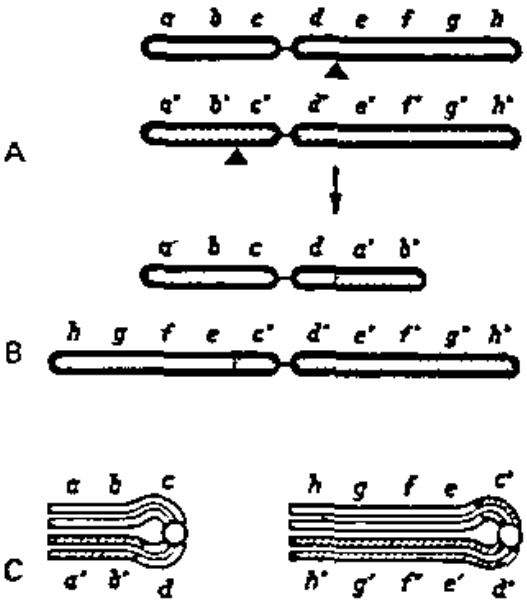


Fig 10 10A–C Diagram illustrating the *origin of pseudoisochromosomes* (*A*) Indication of breakpoints in two homologous chromosomes (*B*) The formation of pseudoisochromosomes after reciprocal translocation at the breakpoints (*C*) Meiotic chromosome pairing of these pseudoisochromosomes (From Rieger et al., 1976)

10.4 B Chromosomes

The term **B chromosomes** was given by Randolph (1928) to a type of chromosome that is present in many plant and animal species and differs in many respects from normal chromosomes, which he termed **A chromosomes.** Other terms for this chromosome type have occurred in the literature such as **accessories, supernumerary,** and **extra chromosomes.** In the present discussion, the term *B chromosome* is preferred over the other terms since it restricts this type to a more well-defined group of chromosomes Since B chromosomes were first discovered in maize (Kuwada, 1925, Longley, 1927), the maize-type of accessory chromosome should be the one that delimits the definition of B chromosomes In maize these chromosomes are distinguishable from normal chromosomes (A chromosomes) according to the following characteristics

1 structure
2 genetic constitution
3 numerical variability
4 meiotic behavior
5 mitotic behavior

B chromosomes in maize are noticeably smaller in size than the normal chromosome set They are about ⅓ of the size of the smallest maize chromosome The centromere of the maize B chromosomes is terminal (Fig 10 11) (Rhoades, 1955) These chromosomes are largely heterochromatic They are also genetically ineffective in that they do not noticeably influence the phenotype of the plant Maize B chromosomes are present in excess to the normal 2n chromosome number of this

Fig 10 11 Diagram of B chromosome of maize in pachytene (From Rhoades, 1955 Redrawn by permission of Academic Press, New York)

species They vary in number between different cells, tissues, individuals, populations, and generations Such B chromosomes do not pair with any of the A chromosomes in meiosis, and they do not pair as regularly among themselves as A chromosomes They have abnormal postmeiotic behavior in that they undergo **nondisjunction** at the second pollen grain division In nondisjunction the two B chromatids do not separate and go to opposite poles but rather stick together and move to the same pole This, in combination with **preferential fertilization,** causes an increase in the number of B chromosomes in the next generation and, thus, in the population Preferential fertilization results in combinations having nonrandom frequencies In this case the male gametes with B's unite more often with the eggs than do those without B's Maize B chromosomes usually are maintained and not lost in the mitotic tissue (Blackwood, 1956) but **mitotic elimination** has been recorded for other plants

The foregoing characteristics shall be the guiding criteria for the classification of B chromosomes in this discussion Deviation in one or the other point will make the classification as B chromosomes more or less questionable If, for example, chromosomes are in excess of the normal somatic chromosome number of a certain species, they definitely should not be classified as B chromosomes Such chromosomes easily fit the description **supernumeraries** or **accessories**

10 4 1 B Chromosome Structure and Genetic Constitution

Since a partial requirement for B chromosomes is their heterochromatin content, many recorded accessories should probably not be included in this category The high content of heterochromatin in B chromosomes must be connected with their genetical ineffectiveness and with the fact that they can accumulate up to a certain limit, at which point they become deleterious Heterochromatin is generally considered to be less genetically active than euchromatin

B chromosomes are generally shorter than A chromosomes and thus susceptible to nondisjunction Since they are considered to be nonessential chromosomes, they seem to undergo morphological changes that do not have any genetic consequences In maize, Randolph (1928) distinguished between B, C, D, E, and F chromosomes, thus designating deviation from the B or standard type Such ***polymorphism*** has also been observed in other plants The most extreme case of B polymorphism has been observed by Matsuda (1970) in *Aster ageratoides* in which 24 morphological deviations from the standard type occur The most characteristic B chromosome shape is the telocentric or acrocentric This is certainly the case in the most thoroughly described types in maize and rye The normal rye chromosome complement (A chromosomes) consists of metacentric and submetacentric chromosomes Thus, the rye B chromosomes are very easily distinguishable by their morphology (Fig 10 12)

In maize, Randolph (1941b) tested 46 linked genes distributed among 17 of the 20 arms of the A chromosomes None of these genes showed disturbed ratios in combination with the B chromosomes This demonstrates that there is complete absence of any known major genes on the B chromosomes

Fig 10 12 Root tip cell of rye with 14 normal A chromosomes and 5 extra B chromosomes Arrows indicate B chromosomes (From Jones 1975 Reprinted by permission of the Academic Press New York)

10 4 2 Numerical Distribution, Variability and Effects of B Chromosomes

In general if a chromosome or a group of chromosomes occur in an even number in all individuals of a species they are not considered to be B chromosomes The number of B chromosomes in maize normally ranges only from 1 to 3 But up to 34 B s have been observed (Rhoades 1955) When the B s ranged from 10 to 15 in number plant vigor was not affected There was a direct correlation between increasing number of B s and vigor seed set and fertility when the B s ranged in number from 15 to 25 Plants with 30 to 34 B s were very low in vigor and entirely sterile In diploid rye ($2n=14$) a maximum number of 10 B chromosomes and in tetraploid rye ($2n=28$) a maximum number of 12 B chromosomes have been recorded (Müntzing 1963)

As mentioned B chromosomes can vary in number among populations among individuals among different tissues of the same individual and between cells of the same tissue Battaglia (1964) summarized this subject In the grass *Poa alpina for instance Muntzing (1948b) found that B s are eliminated from the leaves and* adventitious roots but that they are present in the primary roots the central part and the germ cells In *Sorghum purpureosericeum* Darlington and Thomas (1941) reported that the B s are lost in the roots during seed development that in the growing inflorescence the B s are eliminated from tissues that are not going to produce germ cells but that the pollen mother cells contain a constant number of B s The anther walls and ovaries are intermediate in the amount of loss In the flat worm *Polycelis tenuis* Melander (1950b) found that B chromosomes tend to be lost from the somatic cells of fully grown animals but are retained in the ovarial tissue

On the other hand there are many different species in which the number of B s is very constant within an individual such as in the grasses *Agrostis Alopecurus Anthoxanthum Brıza Dactylis Festuca Holcus Phleum* and *Secale* (Battaglia 1964)

In plants B chromosomes seem to be limited to the angiosperms in which they have been reported in more than 475 species of 163 genera in 42 families (Brown and Bertke 1969) In animals B chromosomes have been reported in flatworms (Melander 1950b) snails (Evans 1960) Isopoda (Rocchi 1967) grasshoppers scale insects Heteroptera Lepidoptera beetles and some Diptera (White 1973) B s seem to be very rare in vertebrates but have been reported in Urodela Reptilia Anura and Mammalia White (1973) published a table with species of animals in which B chromosomes have been recorded

Kodani (1957a 1957b 1958a 1958b) suggested the presence of one or two supernumerary chromosomes in Japanese human populations but Makino and Sasaki (1961 Sasaki and Makino 1965) were not able to confirm this finding

10 4 3 Meiotic and Postmeiotic Behavior of B Chromosomes

B chromosomes are not in any way homologous with A chromosomes but pair with each other However their pairing efficiency is not as high as it is among A chromosomes If B chromosomes are unpaired they can divide at anaphase I at anaphase II or at either one of these divisions depending on the species In most species with B chromosomes their meiotic transfer is normal However in the grasshopper *Locusta migratoria* Rees and Jamieson (1954) observed that the univalent B s lag in the first anaphase spindle and divide tardily causing up to a 20% loss in meiosis Mendelson and Zohary (1972) detected a similar meiotic loss of B s in *Aegilops speltoides* The B remains lagging outside the equatorial plate in anaphase I and then undergoes a precocious division It then fails to be included in the daughter nuclei at the end of the first meiotic division At the end of meiosis the B appears as a micronucleus in 80% to 85% of the pollen mother cells

A search for the cause of B chromosome accumulation in populations of plants and animals has led to the discovery of a **nondisjunction** mechanism Battaglia (1964) distinguished between three major types of nondisjunction in the B chromosomes of plants and a fourth is added here

1 *Secale* type
2 *Sorghum* type
3 *Zea* type
4 *Lilium* type

The **Secale type** of B chromosome nondisjunction results in a postmeiotic preferential distribution of B s It was first discovered by Hasegawa in 1934 and later carefully studied by Müntzing and coworkers for microsporogenesis (Müntzing 1946 1948a Müntzing and Lima de Faria 1949 1952 1953 Lima de Faria 1953) and by Hakansson (1948) for megasporogenesis At the first postmeiotic division the centromere of the B s divides normally but the two chromatids remain closely attached to each other in the regions close to the centromere This has been explained as a stickiness of the heterochromatin of these regions The

two B s then are preferentially directed toward the pole that is responsible for sperm or egg formation *Secale cereale* (rye) is the only species in which such preferential segregation is recorded for micro- and megasporogenesis alike Secale type nondisjunction has also been reported for the male line only in the grasses *Anthoxanthum aristatum* (Östergren, 1947), *Festuca arundinacea, Festuca pratensis Phleum phleoides, Alopecurus pratensis, Briza media Holcus lanatus* (Bosemark, 1957a, 1957b), *Dactylis glomorata* (Puteyevsky and Zohary, 1970), *Deschampsia bottnica D caespitosa,* and *D wibeliana* (Albers, 1972)

In the **Sorghum type** of B chromosome nondisjunction (Darlington and Thomas, 1941), the first pollen grain division is regular, producing a vegetative and a generative nucleus The vegetative nucleus undergoes one or more hastened divisions (called **extra divisions** or **polymitosis,** Beadle, 1933a) giving rise to supernumerary (above the normal two) generative nuclei The results of these divisions is a sterilization of the pollen At the first of such extra divisions, the B's pass undivided to the generative pole Apparently, this division takes place so rapidly that the B's are incapable of dividing

The **Zea type** of B chromosome nondisjunction occurs at the second pollen grain division (Roman, 1947) As already mentioned, this type is coupled with **preferential fertilization.** The generative nucleus possessing the B's unites more frequently (60%) with the egg than does the generative nucleus without the B's (Roman, 1948, Blackwood, 1956)

In the **Lilium type** of B chromosome nondisjunction (Kayano, 1957), the preferential distribution of B's takes place during the first meiotic division of megasporogenesis The nondisjoined B chromosome preferentially passes to the anaphase I pole of the megaspore so that the two B's are present in 75% to 85% of the eggs rather than in 50% This type of nondisjunction also occurs in *Trillium grandiflorum* (Rutishauser, 1956), *Tradescantia virginiana* (Vosa, 1962), *Plantago serraria* (Fröst, 1959), *Phleum nodosum* (Fröst, 1969), and *Cochlearia pyrenaica* (Gill, 1971)

Jones (1975) postulated that most of the systems of B nondisjunction in animals are premeiotic Ehrendorfer (1961) also proposed such a system for the plant *Achillea*

Part VI
Variation in Chromosome Structure

Chapter 11
Chromosome Deletions

Four different classes of structural chromosome changes are being considered in Part VI (Chapters 11–14) deletions, duplications, inversions, and translocations These four classes can be grouped as follows In deletions and inversions the chromosome breaks are confined to one pair of chromosomes only, whereas in duplications and translocations more than one chromosome pair can be involved in chromosome breakage

11.1 Breakage-Reunion and Exchange Hypotheses

All structural changes of chromosomes must be connected in some way or another to chromosome damage and breakage The interpretation of the ultrastructure of such chromosome damage and breakage is very limited by the still persisting ignorance of chromosome fine structure (Chapter 3) A summary by Brinkley and Hittelman (1975) on the ultrastructure of mammalian chromosome aberrations shows the reality of this dilemma They conclude that the actual mechanism involved in the formation of a break or exchange is still in the realm of postulation
Structural chromosome changes are generally considered to depend on **breakage** of chromosomes and on **reunion** of chromosome segments Chromosome breakage results in injured chromosome ends, which differ from natural chromosome ends or telomeres by being **sticky** and having the tendency for reunion with other such injured ends
Most structural chromosome or chromatid changes involve both breakage and reunion Thus, the so-called **breakage-reunion hypothesis** was formulated and put forward by Stadler (1931, 1932), Sax (1938, 1941), Muller (1932, 1938, 1940a, 1940b, Muller and Herskowitz, 1954), Wolff (1961), and by Evans (1962)
According to this hypothesis, breaks occur spontaneously or as a result of mutagens and usually rejoin in the original order by repair processes This phenomenon is called **restitution** (Darlington and Upcott, 1941) If restitution to the original structure does not take place, the chromosomes may undergo structural changes through *the phenomenon of* ***reunion*** *(Darlington and Upcott, 1941), where the broken ends* of the chromosomes or chromatids reunite in a new arrangement If only a single break occurs, the centric fragment may undergo **sister-strand reunion** between the two chromatids Such reunion leads to dicentric chromosomes in the next division

with breakage and further complications in the following cell divisions until loss of the chromosome or death of the cells involved occurs (see discussion of **breakage-*fusion-bridge* cycle**, *Section 11 5*)

Obviously, single chromosome breaks involving loss of larger chromosome segments do not generally produce viable cytogenetic changes, but exceptions may occur For instance, McClintock (*1941a*, *1941b*) observed in maize, that freshly broken ends (1) seemed to "heal" in the sporophyte, (2) did not fuse with other broken ends, and (3) were not subject to sister-strand reunion Since such healing did not occur in the gametophyte, this process is not perpetuated into the next generation Generally a minimum of two breaks must occur to effect change in the karyotype

An alternative to the breakage-reunion hypothesis, is the newer **exchange hypothesis** of Revell (Revell, 1955, 1959, 1960, 1963, 1966, Evans, 1962, Rieger, 1966, Brewen and Brock, 1968) According to this hypothesis, the primary event that leads to chromosome aberrations is not breakage but the formation of so-called **primary lesions** (Fig 11 1A) Such lesions are regions of instability or labile sites

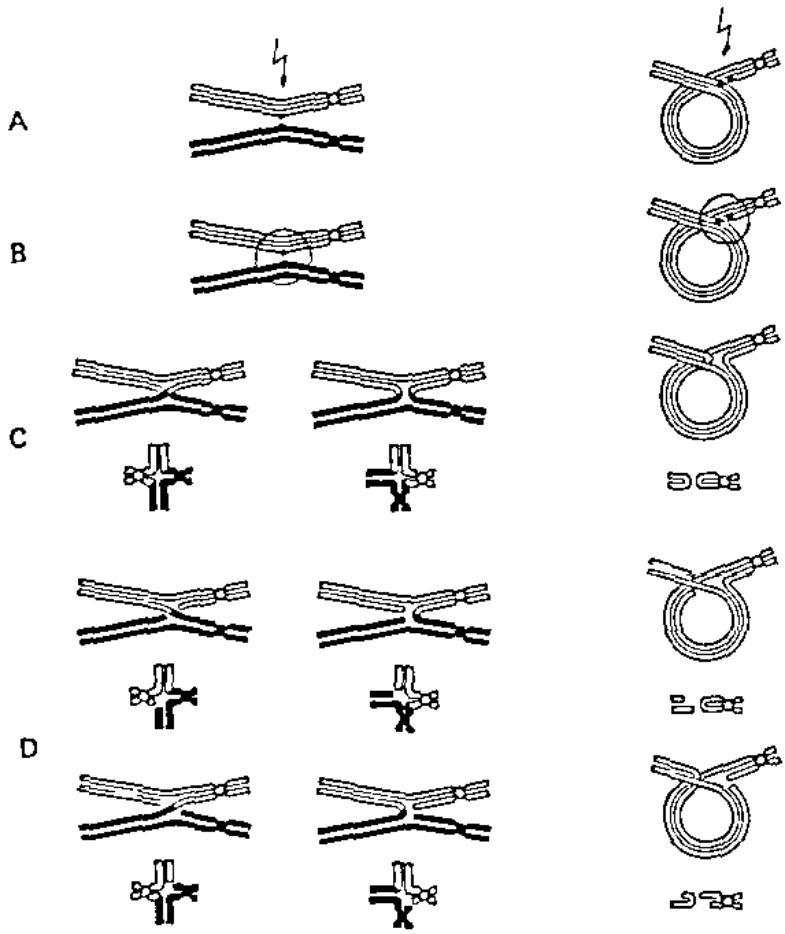


Fig 11 1A–D Diagrammatic representation of the exchange hypothesis (*A*) Primary event primary lesions (*B*) Secondary event exchange initiation (*C, D*) Tertiary event actual mechanical exchange process (Modified after Rieger, 1966)

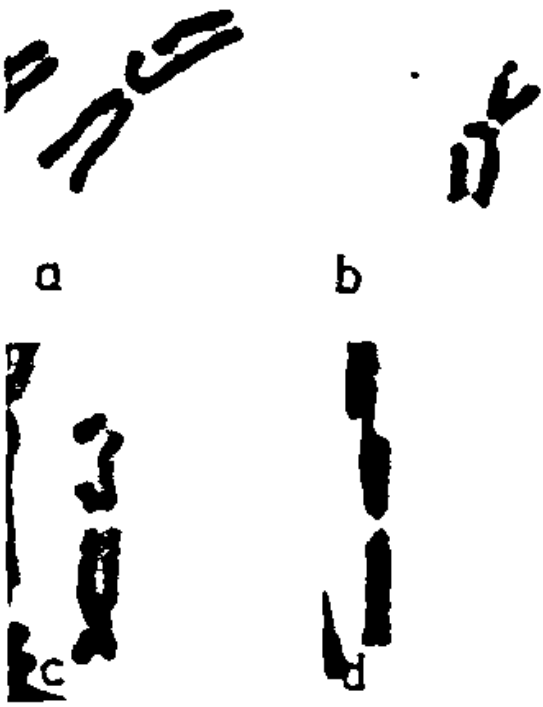


Fig. 11.2a-d Apparent and real chromatid discontinuities in metaphase chromosomes of *Allium*. (*a, b*) Gaps as visible evidence of the primary event, lesions. (*c, d*) Definite displacement of chromosome fragment in real break. (From Kihlman, 1970. Reprinted by permission of Plenum Publishing Press, New York)

in the chromosomes or chromatids. The visible evidence of such lesions are the **gaps** (Figs. 11.2a and b) that are unstained Feulgen-negative regions in the chromosomes or chromatids. Evans (1968) explained that one can observe them at anaphase and see that a chromosome that has such a gap does not lose its fragment. Gaps do not represent discontinuities in the chromosome. Under phase contrast or with the interference microscope, one can actually see a continuity between the two parts of the chromosome on either side of the gap. With a real break (Figs. 11.2c and d), a definite displacement of the fragment is visible, so that according to Evans there is no operational difficulty in distinguishing between gaps and breaks.

The secondary event, according to the exchange hypothesis, is the **exchange initiation** (Fig. 11.1B), an interaction between two lesion sites that is caused by the primary event. Such secondary sites are predisposed to, but have not yet reached actual exchange (Revell, 1959). If the two lesion sites are not close enough together, or are not receptive to each other at the same time, then the two primary lesions fail to interact and may be subject to repair. Consequently, the exchange initiation may or may not be *followed by an actual mechanical exchange process*, which is analogous to crossing over (tertiary event, Figs. 11.1C and D). If this tertiary event takes place, it either leads to a complete (both re-joins occur) or to an incomplete exchange (only one re-join occurs).

The exchange hypothesis postulates that all chromatid rearrangements produced by irradiation are the result of an exchange in which two strands cross one another. It has been developed and supported by a strong group of modern radiation biolo-

gists and is based on experimental evidence Since the discovery of primary lesions or gaps, the actual incidence of chromosome and chromatid breaks is known to be only one tenth of earlier reports (Neary and Evans, 1958 Evans et al., 1959 Revell 1959) Earlier scoring of chromatid breaks was high because of the inclusion of gaps (Thoday 1951) The low frequency of actual breaks and the observation of chromatid exchanges (Fig 11 3), in humans, is good evidence for the exchange hypothesis (Cohen and Shaw, 1964, Brinkley and Hittelman, 1975) Primary lesions are injured sites and possible subchromatid breaks that do not cause discontinuities and may allow the delay of chromatid exchange into the T_1 and T_2 or even later generations (T_1 = one generation after treatment, etc) This is in harmony with observations that have been made particularly after treatment with chemical mutagens Chromosome aberrations were observed several cell generations after treatment by Fahmy and Fahmy (1955) Sluzynska (1963) Evans and Scott (1964), Moutschen (1965) and Müller (1965) If breaks were the immediate result of treatment, such long delay in chromosome rearrangement could not be explained

11.2 Spontaneous and Induced Chromosome and Chromatid Aberrations

As early as 1937, Mather showed that the timing of irradiation determines whether the aberration involves a chromatid or a chromosome If a single radiation event occurs after the S period, only one chromatid will be involved in the lesion If such a radiation even occurs before the S period, both sister chromatids are affected because the lesion becomes replicated with the chromatid
Swanson (1957) believed that all spontaneous aberrations are the result of naturally occurring radiations, but evidence that chromosomal anomalies can be produced by viral infections has accumulated Such breaks and rearrangements in human chromosomes have been reported to be caused by measles (Nichols et al.,

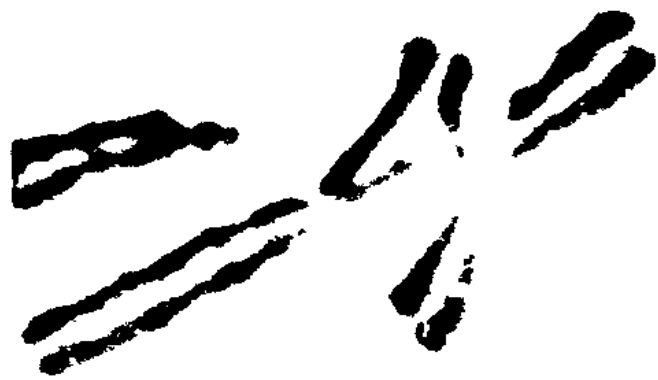


Fig 11 3 Chromatid exchange involving two human chromosomes. (From Cohen and Shaw, 1964 Reprinted by permission of the Rockefeller University Press, New York)

1962) chicken pox (Aula, 1963), meningitis (Makino et al, 1965), and Simian tumor virus SV_{40} (Moorhead and Saksela, 1963) Sometimes, as in the case of SV_{40} infection the sites of the chromosome breakage appear to be nonrandom

The induction of chromosomal aberrations by experimental procedures makes it possible to further inquire into the nature of all those changes that happen spontaneously Methods of inducing chromosome aberrations include the application of various agents such as radiation (Wolff, 1961), chemicals (Shaw, 1970), viruses (Nichols 1970), temperature changes (Hampel and Levan, 1964, Dewey et al, 1971) and mycoplasmas (Paton et al, 1965) A definite correlation between the use of drugs and the increase of chromosome aberrations in the human population has been established by Cohen et al (1967a, 1967b) Novitski (1977) reports that LSD and marijuana have been implicated in chromosome breakage of humans The effects of thiotepa, caffeine, and 8-ethoxycaffeine on the exchange frequency of sister chromatids in *Vicia faba* has been studied by Kihlman (1975)

A number of rare inherited diseases in humans are associated with an increase of chromosome aberrations in cultured fibroblasts and peripheral blood lymphocytes These are the **human chromosome instability syndromes:** Fanconi's anemia (Schroeder et al, 1964, German and Crippa, 1966), Bloom's syndrome (German *et al, 1965, German, 1969), and the Louis-Bar syndrome (Hecht et al, 1966,* Gropp and Flatz, 1967) All three of these syndromes are inherited as autosomal recessives

A method to demonstrate **sister chromatid exchange** (SCE) developed by Latt (1973) has made it possible to quantify the incidence of chromatid breakage Figure 11 4 shows a human lymphocyte cell pretreated according to the **SCE method.** Several researchers demonstrated a close linkage between chromosome aberrations and sister chromatid exchanges in chromosome instability syndromes (Chaganti, 1974, Kato and Stich, 1976 Shiraishi et al, 1976, de Weerd-Kastelein, 1977)

Powerful mutagens such as ionizing radiation (alpha, beta, gamma rays from radioactive sources, x-rays, protons, neutrons) cause only slight increases in SCE frequency (Perry and Evans, 1975) Paradoxically, the effect of some weak carcinogens such as sodium saccharin can be easily measured by the increase in SCE's (Wolff and Rodin, 1978) A small but significant rise in the number of SCE's was observed after the exposure of fresh human lymphocytes to 30 minute treatments with diagnostic ultrasound (Liebeskind et al, 1979)

Chromatid-type damage, like **triradial chromosomes**[1] and chromosome-type damage, like dicentrics, were observed in Louis-Bar syndrome lymphocytes by Taylor et al (1976) after radiation with x-rays in the G_0 phase The hypothesis is that there is a defect of DNA repair in these patients that leads to radio-sensitivity According to this hypothesis, Louis-Bar syndrome lymphocytes lack the full complement of functional polynucleotide ligases that are able to join breaks in one strand of a DNA double helix

[1]Triradial chromosome a three armed chromosome configuration involving two nonhomologous chromosomes arising from an interaction between an **isochromatid break** and a chromatid break

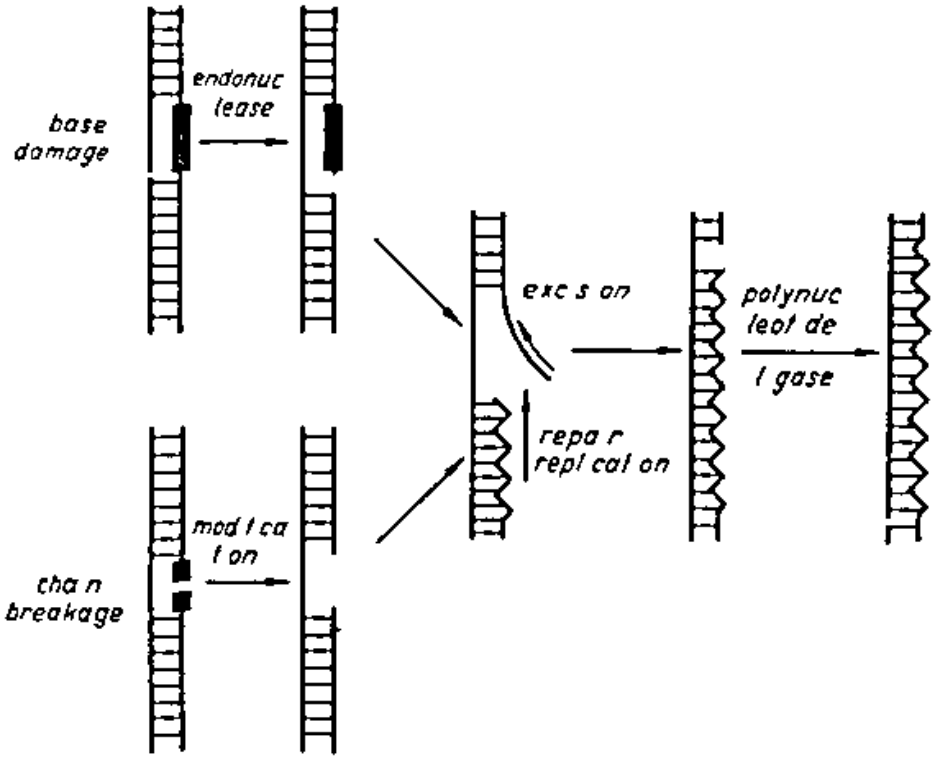


Fig 11 5 Schematic representation of exc sion repair of damaged DNA bases and broken strands involving repair replication (From Cleaver 1974)

ases Such polymereases are part of an **enzymatic proofreading mechanism** (Goodenough 1978) that functions during chromosome replication to compensate for spontaneous and induced mutations This mechanism provides for the insertion of nucleotides into the damaged DNA It may occur after the excision of damaged DNA fragments (**excision repair,** Fig 11 5) Such **repair replication** has been observed in eukaryotes after treatment with x rays UV light and chemical mutagens but it is not known in prokaryotes after ionizing radiations Excision repair is generally considered to produce high fidelity **Postreplication repair** (Rupp and Howard Flanders 1968) however is considered to be error prone It does not act on primary lesions but on secondary lesions that originate as a consequence of unrepaired primary lesions Streisinger et al (1966) developed a model of such **misrepair** according to which after DNA breakage the DNA strand may **buckle,** and DNA strands that are either too long (addition) or too short (deletion) may be synthesized

Drosophila became a classical cytogenetic object for the study of x ray induced aberrations The large size of salivary gland chromosomes of *Drosophila* allowed a very exact study of small and larger chromosome aberrations Since the Morgan school had already made available a great amount of genetical data in *Drosophila* by this time (Chapter 1) the cytological changes could be easily checked against and correlated with this genetic information Muller reported x ray induced translocations and other chromosome aberrations in *Drosophila* as early as 1927 In 1929 they were demonstrated cytologically by Painter and Muller During the following decades the early results in *Drosophila* were confirmed in many plant and

animal species, and by 1946 (Catcheside et al.) a classification system for the description of different chromosome aberrations in *Tradescantia* was completed. The meiotic chromosomes of *Tradescantia* and the mitotic chromosomes of *Vicia faba* were also ideal materials for the study of chromosome aberrations induced by radiation and chemicals. The large size of the chromosomes, the small number of them, and the ease of obtaining large numbers of cells for comparison made these species ideal objects for such investigations.

Induction of mutants by radiation has been used as a tool in plant breeding. According to a report of the International Atomic Energy Agency (IAEA), 68 useful mutant varieties of food and crop plants were released to farmers between the period from 1930 to 1971 (Nabors 1976). Factors such as chemicals, infrared rays, moisture, temperature, and oxygen applied to the living plant tissue before, during, and after radiation change the effect of the radiation treatment (Nilan 1956).

Berns et al. (1979) have demonstrated that the laser microbeam can be used to produce heritable deficiencies on preselected regions of individual chromosomes. They conducted extensive studies on the ribosomal genes of salamander (*Taricha*) and rat kangaroo (*Potorous*) cells in culture. These cells were chosen because they remain flat during mitosis, making all chromosomes easily identifiable during mitosis. Most human cells, for instance, round up during division (Berns 1978).

11.3 Terminal Deficiencies

Bridges (1917) defined a **deficiency** as "a structural change of a chromosome resulting in the loss of a terminal acentric chromosome-, chromatid-, or subchromatid segment and in the loss of the genetic information which this chromatin segment contains." Bridges' work (1923) on structural changes in *Drosophila* chromosomes was briefly mentioned in Chapter 1. In its classical sense, a deficiency is of a terminal nature and involves only a single chromosome break followed by a healing of its broken end (Fig. 11.6A). In contrast, a **deletion** involves an intercalary chromosome segment and requires two chromosome breaks (Fig. 11.6B). However, in practice, the term "deletion" frequently is used for both of these types of structural chromosome changes. Generally, in both instances a centric and an acentric chromosome segment are produced. The centric segment will persist during cell division, while the acentric fragment will be lost. Most chromosome aberrations that cause large deficiencies will lead to the death of the cell involved or will, at least, prohibit sexual reproduction. They are eliminated from the population and will not survive or become part of a permanent karyotype.

The terminal deficiency type of chromosome aberration is a category by itself since it does involve only one break. Therefore, a tendency for sister strand reunion (Section 11.1) of chromatids or reunion of broken chromosome ends exists which does not permit stability. Different tendencies for such sister strand reunion have been observed depending on biological material, treatment, or chromosome material involved. Healing of broken chromosome ends can occur in plants, but it seems to be very rare in animals. Simple chromosome breaks in *Drosophila* and other ani-

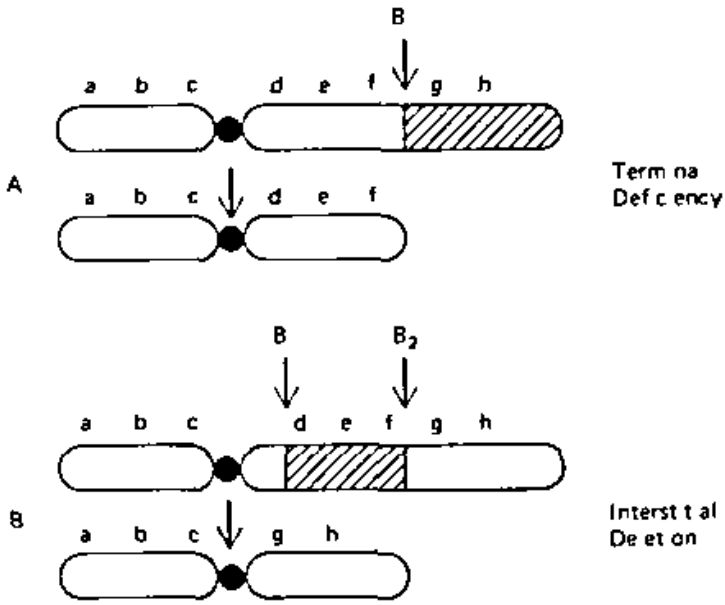


Fig 11 6A and B Illustration of deficiency and deletion (*A*) A terminal deficiency caused by a single chromosome break (*B*) An interstitial deletion caused by two chromosome breaks

mals generally are not stable because of sister strand reunion In plants the treatment seems to make a difference If maize was treated with ultraviolet radiation terminal deficiencies mainly resulted If x rays were applied only interstitial deletions were observed (Stadler 1941 Stadler and Roman 1948)

If the break occurs in the heterochromatic portion of the chromosome it is more likely to heal than if it happens in the euchromatin White (1956) and Southern (1969) reported after studying the centromere in grasshoppers that simple breaks through the centromere were stable throughout the spermatogonial mitosis Centromere regions are generally heterochromatic Khush and Rick (1968a) observed that the frequency of recovered x ray induced breaks is highest in heterochromatin and lowest in euchromatin

The extent to which a terminal deficiency can be tolerated in animals has been tested in *Drosophila* Demerec and Hoover (1936) have shown several deficiencies that demonstrate the loss of the left tip of the X chromosome (Fig 11 7) Up to 11 bands are involved in this terminal deficiency including such loci as *y ac* and *sc* If only 8 bands in this region are lost this deficiency is lethal to the whole organism in the homozygous condition but not to individual cells A loss of 4 bands is viable in the homozygous as well as in the hemizygous condition If one considers that the X chromosome of *Drosophila* has more than 1000 bands and that the entire chromosome complement consists of approximately 5000 bands then a loss of 8 bands seems to be minimal but nonetheless consequential On the other hand loss of heterochromatic segments can occur almost unnoticed Large pieces of the Y chromosome of *Drosophila* may be deficient without any lethal effect The effects of hemizygous deletions and that of duplications that cover the entire autosomal complement of *Drosophila* were reported by Lindsley et al (1972) Aneuploidy (Section 16 2) in 57 **dosage-sensitive loci** leads to recognizable changes in the organism

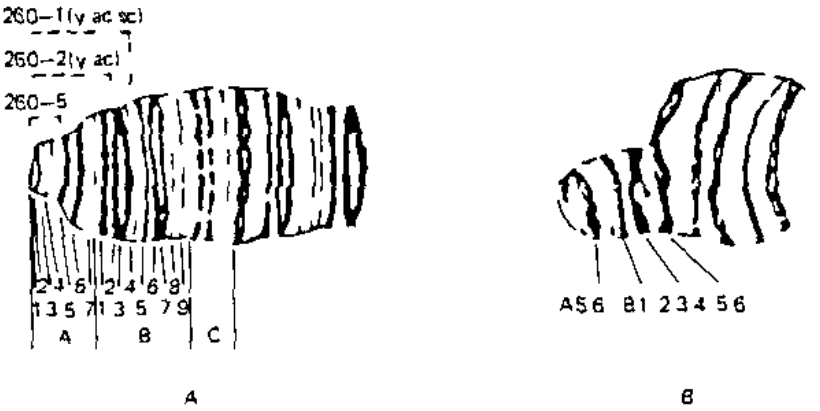


Fig 11 7A and B Deficiency at tip of X-chromosome of *Drosophila melanogaster* (*A*) Normal tip (*B*) Deficiency of 10 or 11 bands (260-1) which includes the genes *y ac* and *sc* (From Demerec and Hoover 1936 Redrawn by permission of American Genetic Association Washington D C)

11 4 Interstitial Deletions

The loss of an intercalary or interstitial chromosome segment is referred to as a **deletion** Painter and Muller in 1929 described the parallel cytology and genetics of such induced deletions in *Drosophila* During the same year Serebrovsky suggested on the basis of x ray experiments that perhaps all mutations are deletions or other chromosome aberrations It is now known that deletions can vary from the absence of a single nucleotide to large chromosome segments It is hard to determine where point mutations end and where deletions begin The genetic proof for a deletion is its failure to back mutate to the original form and to recombine in genetic crosses with two or more point mutations that do back mutate and recombine

McClintock (1938b) succeeded in the phenotypical demonstration of an interstitial deletion in maize that could be confirmed cytologically This deletion is exceptional in that the deleted portion is centric rather than acentric The chromosomal region involved included the locus of the gene Bm_1 in the short arm of chromosome 5 close to the centromere The recessive allel bm_1 of this gene expresses brown midrib, producing a brown color in lignified cell walls The absence of Bm_1 as well as bm_1 (interstitial deletion) also produces brown midrib The heterozygous condition (Bm_1/bm_1) causes variegated tissue The deficiency was caused by x raying pollen containing a normal haploid chromosome complement with the dominant gene Bm_1 By placing this pollen on silks of recessive plants (bm_1 bm_1), two plants, variegated for Bm_1 and bm_1 were found in a progeny of 466 plants The cytological analysis of the variegated plants revealed an interstitial deletion in one homologue of chromosome 5 and a small ring shaped chromosome (Fig 11 8A) The deletion chromosome and the ring chromosome arose as a result of two breaks in the normal chromosome 5, one dividing the centromere, the other breaking the chromosome at a distance from the centromere of 1/20 (plant 1) or 1/7 (plant 2) of the total chromosome length Proof for the assumption that the

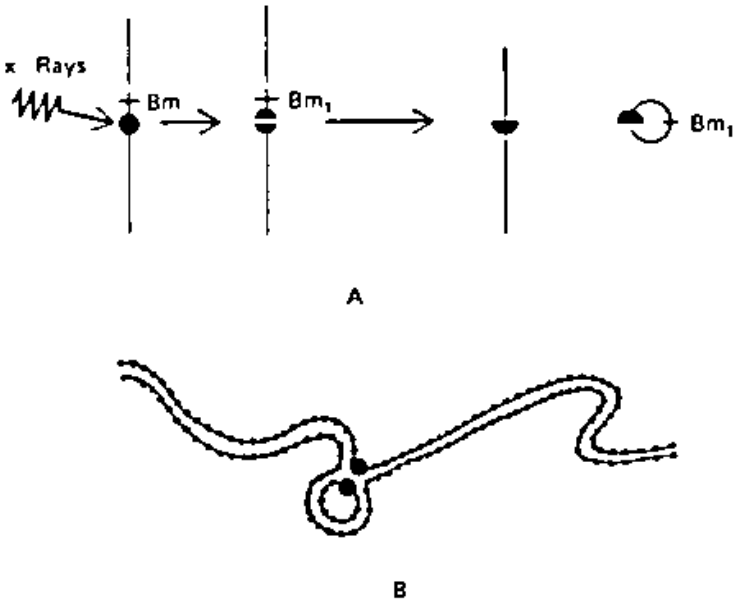


Fig 11 8A and B Interstitial deletion in the short arm of chromosome 5 of maize involving locus Bm_1 causing color change in the midrib of leaf sheath and blade particularly in older leaves (*A*) Demonstration of chromosome breakage by x rays producing a deleted centric rod chromosome and a small ring shaped chromosome (*B*) Synaptic pachytene configuration of normal deleted and ring shaped chromosomes

ring-chromosome was indeed the region that was missing in the deletion chromosome was the discovery of the synaptic configurations in pachytene between normal, deleted, and ring-chromosomes (Fig 11 8B) It was discovered that the ring chromosome could get lost from certain portions of the somatic plant tissue and that such portions would show brown streaks (variegated) Thus, it was assumed that the dominant Bm_1 locus was carried on the ring Another phenomenon discovered in connection with these ring-chromosomes was the **breakage-fusion-bridge cycle** described in the next section

11.5 Breakage-Fusion-Bridge Cycle

McClintock (1938a, 1938b, 1941a, 1941b 1941c, 1942, 1944) found that the size of these ring-chromosomes changed through successive nuclear cycles In order to change its size, the ring must obviously break Figure 11 9 shows how ring chromosomes change size in somatic tissue It should be remembered that breakage was the original event that led to the formation of the ring (fusion). It is understood all along that the ring chromosome has a centromere If the ring reproduces itself in interphase and no sister strand crossing over occurs in prophase, then the two ring chromatids can separate from each other in anaphase without difficulty, reproducing two new equally sized ring-chromosomes that do not differ in size from the original one However, if sister chromatid exchange (breakage + fusion) occurs in prophase, a ring of twice the size will be produced initiating the cycle The ring will have two centromeres Such a dicentric ring will behave like any other dicentric chromosome in that the two centromeres will move toward opposite poles in

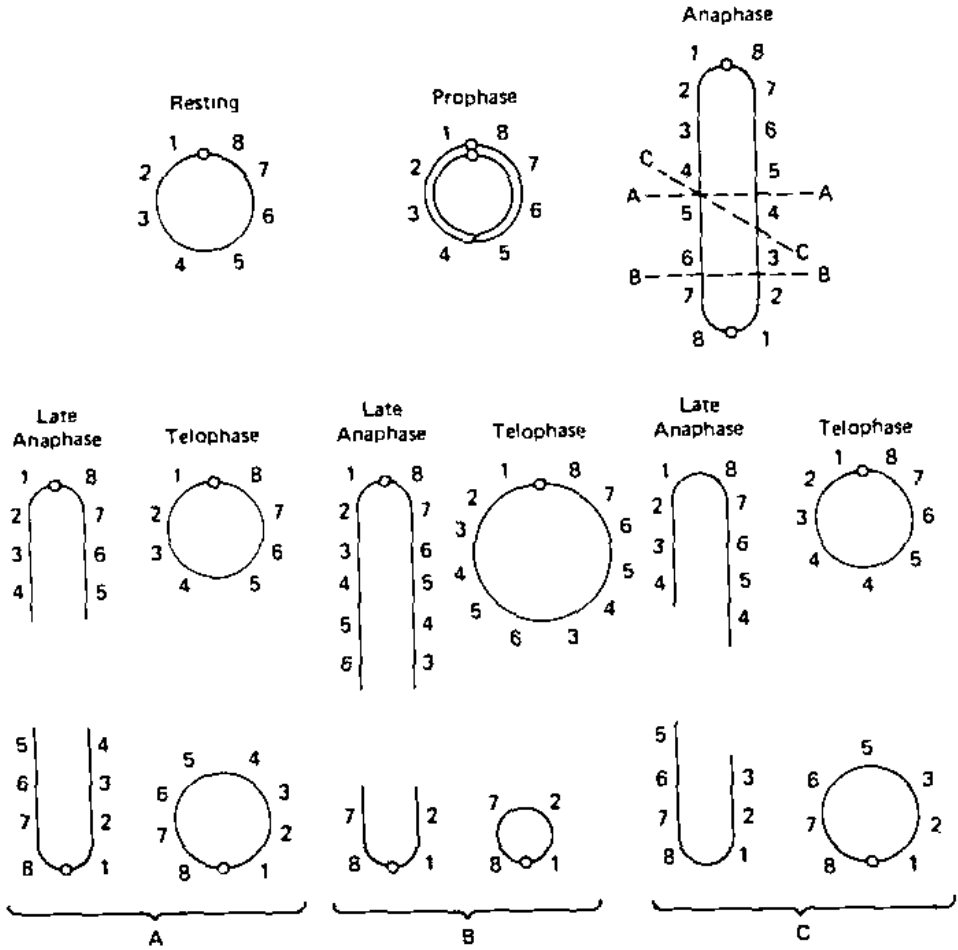


Fig 11 9 Breakage fusion-bridge cycle as observed by McClintock (1941) in maize In the upper portion of the illustration, the drawing on the left is a ring chromosome in an undivided resting state, the middle one is a replicated ring with a "crossover" between sister strands, and on the right is a drawing of a double sized dicentric ring in an anaphase with A, B and C representing three possible breakage situations The bottom half rep resents the results of the three possibilities at late anaphase and telophase (After McClintock, 1941b Redrawn by permission from Genetics Society of America, Austin, Texas)

anaphase and will form an anaphase double bridge Chromosome breakage in anaphase will occur subsequently and will complete one turn of the breakage-fusion bridge cycle The double sized ring can break at different points along the ring-chromosome Three possible different breakage situations (A, B, C) are shown in Fig 11 9 The result are rings of different sizes in the next nuclear cycle, which stem from the fusion of the broken chromosome ends If the different segments of the anaphase double sized ring chromosome are numbered (Fig 11 9), then it becomes obvious that duplications and deficiencies result from the uneven breakage of the ring The cycle is initiated by **primary chromosome aberrations** (deletions) and results in **secondary chromosome aberrations** (deletions and duplications) The smaller rings that occur as a result of this cycle often are lost from the tissue It is obvious in Fig 11 10 that there is an alternation between breakage fusion

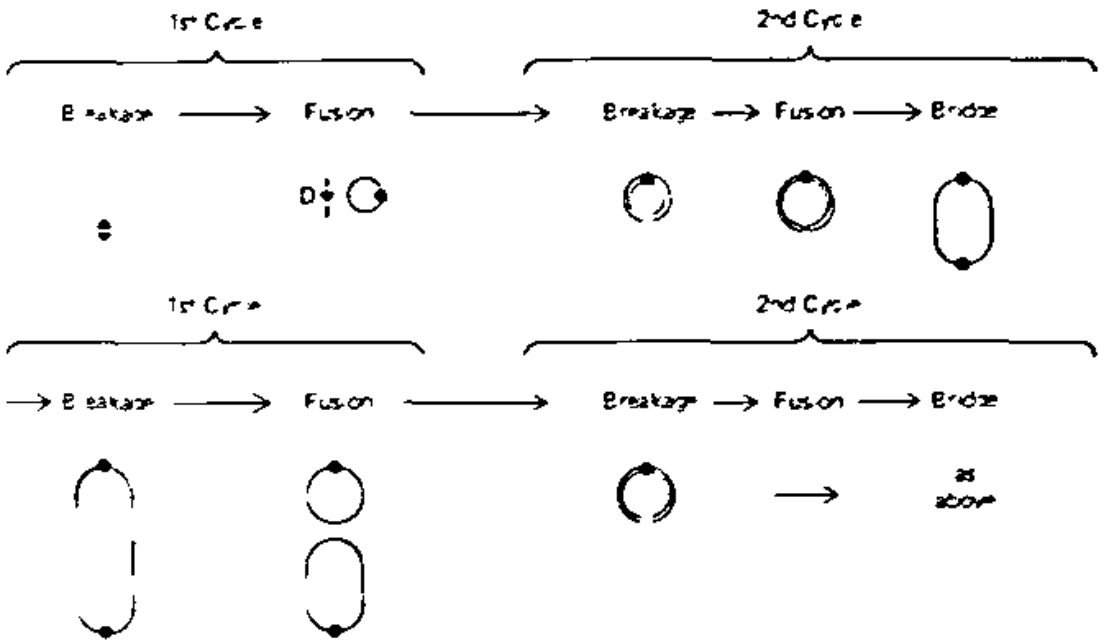
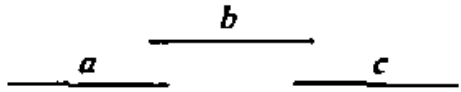


Fig 11.10 Demonstration of alternation between breakage fusion cycles and breakage-fusion-bridge cycles.

cycles and breakage-fusion bridge cycles. Other examples of breakage fusion-bridge cycles exist and do not necessarily involve ring-chromosomes.

11.6 Genetic and Cytological Tests of Deletions

Deletion mapping or **cytogenetic mapping** has already been mentioned in Chapter 4 as one of several possible ways of locating genes on chromsomes in *Drosophila* (Mackensen, 1935, Slizynska, 1938). Deletion mapping in its strict sense (Rieger et al., 1976) is the genetic localization of the positions of deletions in the linkage structures of eukaryotes and prokaryotes. This kind of mapping is based on three mutants (*a*, *b*, and *c*) that differ from the wild-type by a deletion being tested for recombination. If two mutants (*a* and *c*) mutually recombine and yield the wild-type but neither recombines with the third (*b*), then the three deletions are overlapping in the order *a*, *b*, *c* in the following fashion.



Deletion mapping also has been successfully applied in viruses by Benzer (1955) and in bacteria by Ames and Hartman (1963). It also may be useful for chromosomal localization of autosomal genes in humans (Nance and Engel, 1967).

Cytogenetic deletion mapping has recently been possible in bacteriophages that have much simpler genomes than the genomes of complex higher organisms. In bacteriophage, many mutants are readily available in which practically any region of the genome is deleted or replaced by nonhomologous DNA derived from the bacterial host or from other phages. These deletions can be used as genetic markers

and mapped genetically if they are not lethal If by the DNA hybridization method (Harris and Watkins 1965 Chapter 1) normal and deleted DNA genophores were combined to form double helices a loop was formed at the site of the deficiency (Fig 11 11) These **heteroduplex genophores** look remarkably similar to maize pachytene chromosomes (Westmoreland et al 1969) An example of a heterozygous deletion in a *Drosophila* salivary gland cell is shown in Fig 11 12
Burnham (1962) suggested the possible use of deletion chromosomes in locating recessive genes Smith et al (1968) extended this principle for the possible localization of the gene for **cystic fibrosis** (*cf*) of the pancreas to the short arm of chromosome 5 in humans (Fig 11 13) A patient with a heterozygous short arm deletion for chromosome 5 has only a single set of genes on the homologous segment of the missing piece (hemizygosity) Should this single set of genes contain a recessive

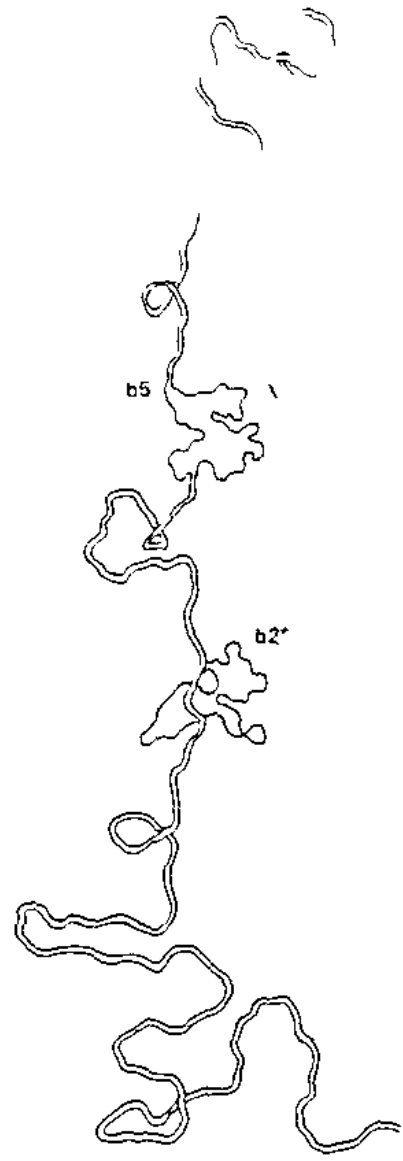


Fig 11 11 A drawing of an electronmicrograph of viral genophore aberrations produced by heteroduplex formation A loop was formed at the site of the deficiency (b2+) where normal and deleted DNA genophores were combined to form double helices (From Westmorland et al 1969 Redrawn by permission of the American Association for the Advancement of Science Washington D C)

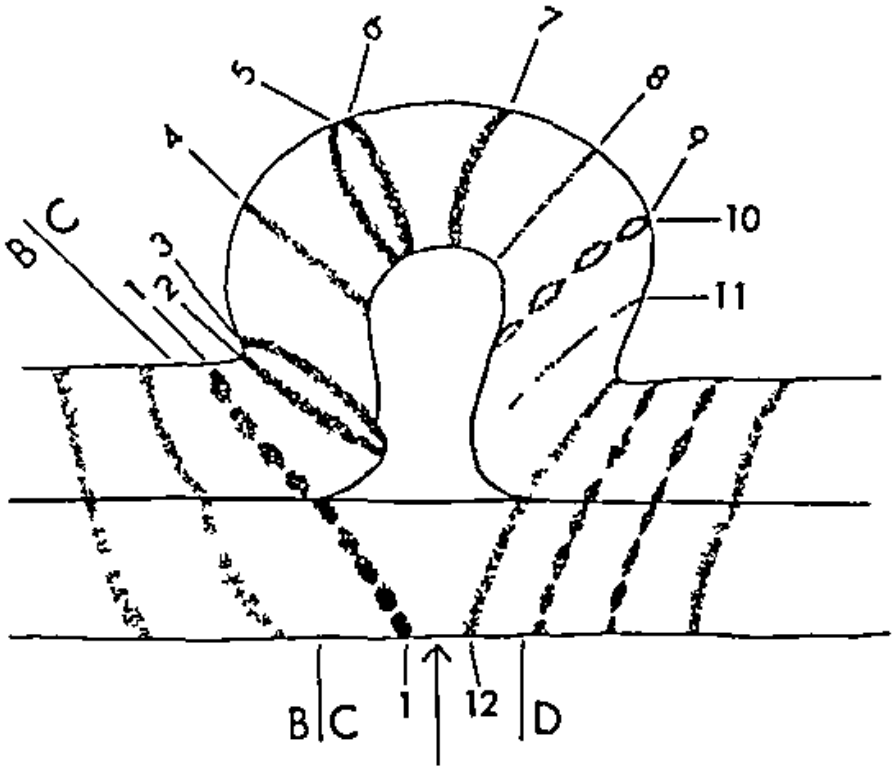


Fig 11 12 Diagram of heterozygous deletion in a *Drosophila* salivary gland cell (From *Principles of Human Genetics Third Edition by Curt Stern W H Freeman and Company* Copyright © 1973)

cf gene the patient would express cystic fibrosis since there would be no normal allelic gene to counteract the adverse effect of the mutant gene

11 7 Human Deletion Syndromes

In 1963 Lejeune et al for the first time could link a clinical syndrome to a chromosome deletion in humans They discovered that the loss of a short arm segment in chromosome 5 (5p-) of the B group (see Fig 2 3) resulted in an abnormal cry of the affected baby resembling that of a suffering cat This phenomenon results from a malformation of the larynx It is also called the **cri du chat syndrome** Other symptoms associated with this syndrome are severe facial malformations and microcephaly and above all mental retardation The severity of this syn

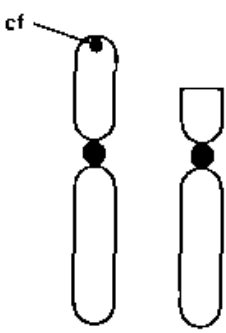


Fig 11 13 Possible detection of gene for cystic fibrosis (cf) in short arm of human chromosome 5 by way of deletion chromosome

Fig. 11.14 Quinacrine fluorescent karyotype of a C group deletion in human chromosome 9 [46, X/Y, del(9) (pter—p22)] (Courtesy of Dr. Penelope W. Allderdice, Faculty of Medicine, Memorial University, St. John's, Newfoundland, Canada)

drome varies from patient to patient and is thought to depend on the extent of the deletion. Many instances of this syndrome have now been demonstrated.

Another deletion syndrome in the human B chromosome group involves the short arm of chromosome 4 (4p-) (Wolf et al., 1965a, 1965b, Wolf and Reinwein, 1967, Hirschhorn et al., 1965, and others). This syndrome seems to occur much less frequently than the 5p- syndrome. None of the children with 4p- seem to have the characteristic cat cry. They are much more grossly malformed than the 5p- subjects, and facial anomalies are similar to the chromosome 5 deletion.

The possibility of a distinct 13 q- deletion syndrome that involves the D group of human chromosomes was postulated by Allderdice et al. in 1969. This conclusion was based on their own observations as well as on earlier similar findings (Gey, 1967, Laurent et al., 1967, Mikelsaar, 1967). The deletion involved about 20% of the long arm of chromosome 13. The syndrome resembles anomalies that were earlier described for D ring chromosome cases. It is characterized by microcephaly; eye, ear, and nose abnormalities, marked facial asymetry; and the absence of thumbs. By 1977 (Nielsen et al.), ten cases of deletion syndrome involving the

long arm of chromosome 13 were known in humans Three of them were terminal deficiencies, four were interstitial deletions, and three were unspecified cases of 13 q- Only one case (described by Nielsen, et al , 1977) involved a person older than two years, a 65-year-old mentally retarded woman with a karyotype 46, XX, del (13) (q21-q31)

Alfi et al (1973, 1974) and Allderdice et al (1976) described four cases of C group deletion in chromosome 9 [46, del (9)(pter-p 22)] A karyotype of this syndrome is presented in Fig 11 14 The most striking facial feature of this syndrome was trigonocephaly (flat and triangular head) (Fig 11 15) A deletion in the long arm of one of the 3 E group chromosomes (17q-, 18q-, Fig 2 3) was first detected by De Grouchy et al in 1964 Lejeune et al (1966) found two cytologically and clinically similar cases and thus established this Eq- syndrome The clinical observations associated with this syndrome included mental retardation, growth and development failure, microcephaly, anomalies of ears and eyes, and genital abnormalities in males Curran et al (1970) showed a patient with several of these but lacking genital abnormalities Deletions in the short arm of chromosome 18 (E group) have been found repeatedly but such an 18p- deletion could be associated with a syndrome only in about 50% of the observed cases (Ferguson-Smith, 1967)

A G group deletion syndrome was discovered in 1960 by Nowell and Hungerford It is often related to the *Philadelphia* or Ph¹ chromosome Originally, chromo-

Fig 11 15 Frontal view of proband with chromosome 9 deletion syndrome exhibiting trigonocephaly (From Allderdice, P W et al 9 pter → 22 deletion syndrome A case report In Bergsma, D , Schimke, R N (eds) "Cytogenetics Environment and Malformation Syndromes ' New York Alan R Liss for March of Dimes Birth Defects Foundation, BD OAS XII (5) 151–155, 1976).

some 21 was thought to be involved but it is not known which of the two G group chromosomes are deleted The deletion involves about 61% of the DNA of a normal G group chromosome (Gq-), which is most of the long arm The abnormal chromosome is usually found in heterozygous condition in blood and marrow cells of chronic myeloid leukemia (CLM) patients In tissues other than the hemopoietic system (responsible for blood cell production), the chromosomes are generally normal (Tough et al , 1961) Several cases with two Gq- chromosomes (homozygous deletion) have been found and seemed not to affect the characteristics of the disease (Dougan and Woodcliff, 1965) The discovery of the Gq- syndrome was rapidly confirmed by another research team (Baikie et al , 1960, Tough et al , 1961) The positive evidence of a direct linkage between the Gq- chromosome and CML was not immediately obvious, but by 1971, Hamerton could state that there is little doubt that most, if not all, adequately diagnosed cases of CML carry the Ph^1 chromosome

Deletions that involve an X chromosome have been reported by several authors (Jacobs et al , 1960, Fraccaro et al , 1960, Hamerton, 1971b) These deletions can involve the short arm (XXp-) or the long arm of the X chromosome (XXq-) Since the Turner syndrome (Chapter 16) in humans seems to be determined by the hemizygous condition of the short arm of the X chromosome, individuals with XXp- express this sex anomaly This syndrome is also referred to as **ovarian disgenesis** (or female infertility) The XO Turner syndrome is usually chromatin-negative, while the XXp- condition is chromatin-positive in that the deleted X usually forms a small Barr body According to Hamerton (1971b) only about 10% of all chromatin-negative individuals are mixoploid (e g , 45, X/46, XX), while over 80% of all chromatin-positive individuals are mixoploid (e g , 46 XXp-/46XX)

Several deletions in the satellites of human chromosomes (groups D and G) have been observed that were not associated with syndromes The satellites are considered to be heterochromatic A loss of chromatin in these regions obviously is not of any genetic consequence Ferguson Smith and Handmaker (1961) showed that the manifestation of satellites varies from cell to cell

Chapter 12
Chromosome Duplications

12.1 Types of Chromosome Duplications

A duplication is a structural change in chromosomes that causes doubling of a chromosome segment. The size of the doubled segment can vary considerably. Chromosome duplications are generally more tolerated by an organism than chromosome deletions.

Duplications can occur within a chromosome or among nonhomologous chromosomes and consequently are called **intrachromosomal** or **interchromosomal** duplications. According to Swanson (1957) *there are three types of duplications*:

1. tandem duplications
2. reverse tandem duplications
3. displaced duplications

The first two duplication types are intrachromosomal, the third is interchromosomal. Figure 12.1 illustrates these three types. The chromosome segment "def" is the duplicated segment in all three cases. Modifications of the first two examples (A, B) can occur if the duplicated segment is shifted to a different position in the same arm or in the other arm. Duplications may occur in different ways. McClintock (1941a, 1944) demonstrated how meiotic pairing and crossing over in a **reverse tandem duplication** heterozygote can initiate a breakage-fusion-bridge cycle (Section 11.5). The duplication involved the short arm of chromosome 9 of maize and included genes for *colorless aleuron* (*C* 26), *shrunken endosperm* (*sh* 29) and *waxy pollen* and endosperm (*wx* 59) (Fig. 12.2). Pachytene association of chromosomes in duplication heterozygotes led to two possible ways of pairing. In one instance the reverse tandem chromosome segment folded up and resulted in pairing within the same chromosome. Another way of pairing involved both homologous chromosomes, as shown in the figure. The result of this pairing was a bridge and a fragment in anaphase I. Depending on where the break in anaphase I would take place, the resulting gametes would carry smaller or larger deficiencies or duplications. The tendency for **sister chromatid reunion** at broken chromosome ends would lead to a new breakage-fusion-bridge cycle during the next mitotic division (Fig. 12.2).

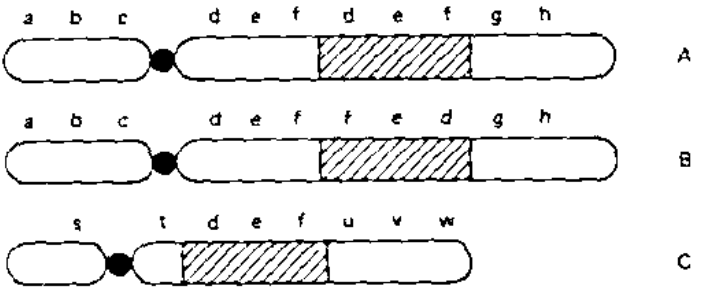


Fig 12.14–C Drawing of the three types of chromosome duplication (*A*) tandem duplication (*B*) reverse tandem duplication (*C*) displaced duplication

12.2 Origin of Chromosome Duplications

Chromosome duplications can generally occur in three different ways (Rieger et al 1976)

1 by primary structural changes of chromosomes
2 by unequal crossing over of chromatids
3 by crossing over in inversion or translocation heterozygotes (Chapters 13 and 14)

Primary structural changes resulting in duplications involve three breaks (B B and B_3 Fig 12.3) Two breaks (B and B) in a normal chromosome (Fig 12.3A) result in a deleted centric chromosome (abc ghi) and an acentric fragment (def) (Fig 12.3B) The third break (B_3) could occur in the homologous partner chromosome (Fig 12.3C) If the fragment is inserted into the partner chromosome a ***tandem duplication*** results (Fig 12.3D)

The result of such an **interchromosomal transposition** is a deficiency-duplication individual If such an individual mates with a normal one a duplication heterozygote could result that has a normal and a duplication chromosome (Fig 12.3D)

The possible origin of such primary structural changes has been described in a unique way by McClintock in maize She called it the **Activator-Dissociation system** (see Chapter 1) Since this system involves a type of **position effect,** it will be discussed in more detail under that heading in this chapter (section 12.3)

Chromosome duplication as a result of **unequal crossing over** is illustrated in Fig 12.4 Usually crossing over occurs between homologous chromatids at loci that exactly correspond to each other in gene content (alleles) Such loci are responsible for the same biochemical and developmental processes When the mechanism of pairing (Section 7.2.2) and crossing over (Section 4.2) is less specific chromosome aberrations can occur as a deviation from the normal process Such less specific pairing is observed particularly in areas in which heterochromatic chromosome segments are involved Riley and Law (1965) called this **heterochromatic fusion** or **nonspecific pairing,** depending on the type of chromatin involved The cytological evidence of nonspecific pairing was given by McClintock (1933) during her studies of maize pachytene

Unequal crossing over was first observed in *Drosphila* by Sturtevant in 1925 involving the well known *Bar* locus (*B* chrom 1.57 0) It produces one chromatid containing a chromatid segment twice (duplication) and the second lacking that chromatid segment (deletion) In Fig 12.4A two normal homologous chromo-

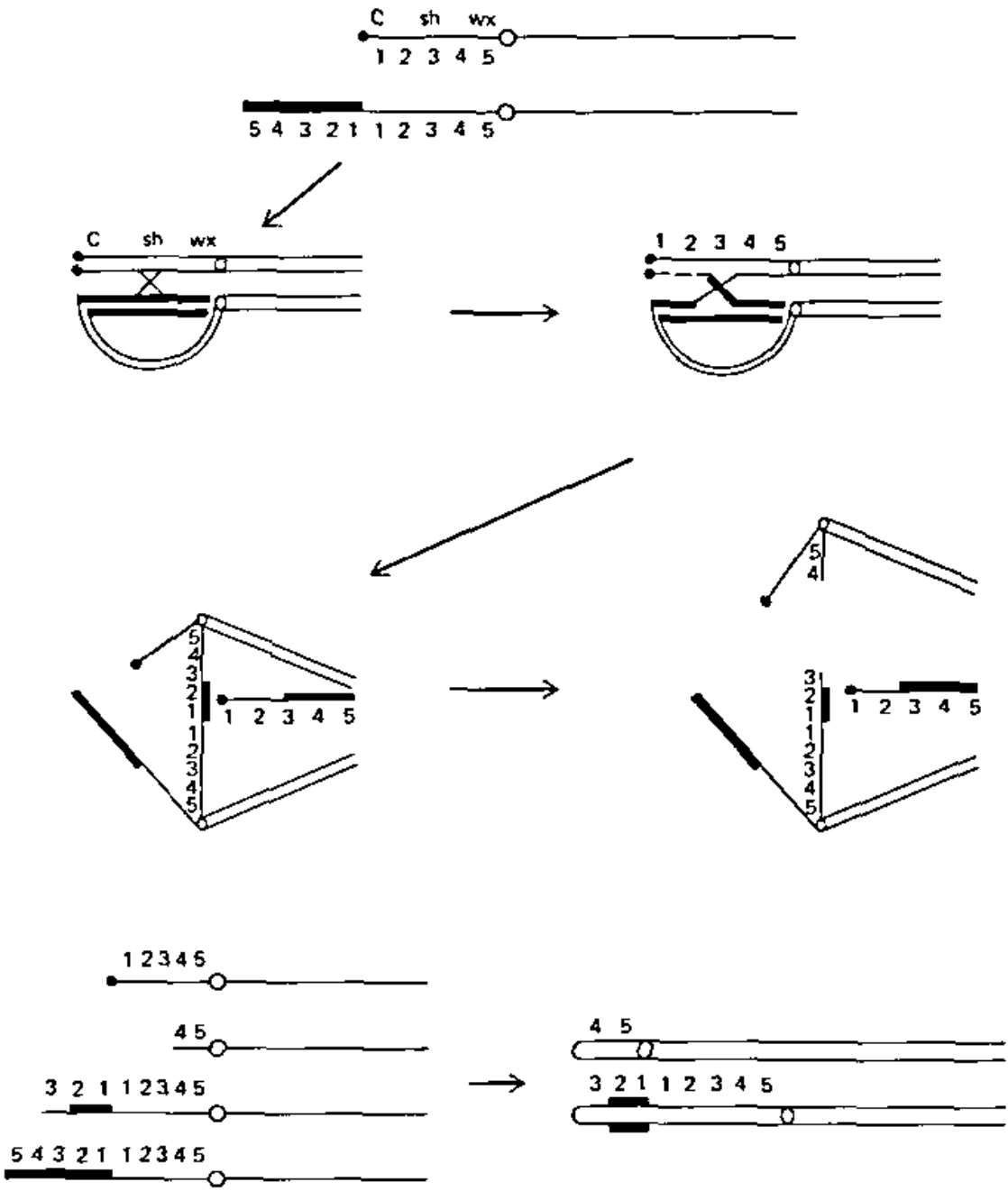


Fig 12 2 Diagram of a **reverse tandem duplication** in the short arm of chromosome 9 of *maize initiating a breakage fusion bridge cycle Explanation in text (Modified from* McClintock, 1941a Redrawn by permission of the Genetics Society of America Austin Texas)

somes are shown with breakpoints (B_1 and B_2) indicated in two non sister chromatids (regions fg and cd) Figure 12 4B shows the four chromatids after crossing over is accomplished *Figure 12 4C shows the four resulting chromosomes that* will be distributed to the four gametes Two of them are normal (abc defghi) one duplicated (abc defdefghi) and one deleted (abc ghi)

The formation of an abnormal hemoglobin (Hb-Lepore) in man was postulated by Baglioni (1962) as a result of the process of unequal crossing over and deletion The *Bar* duplication in *Drosophila* will be discussed in more detail because it gave rise to the concept of the position effect

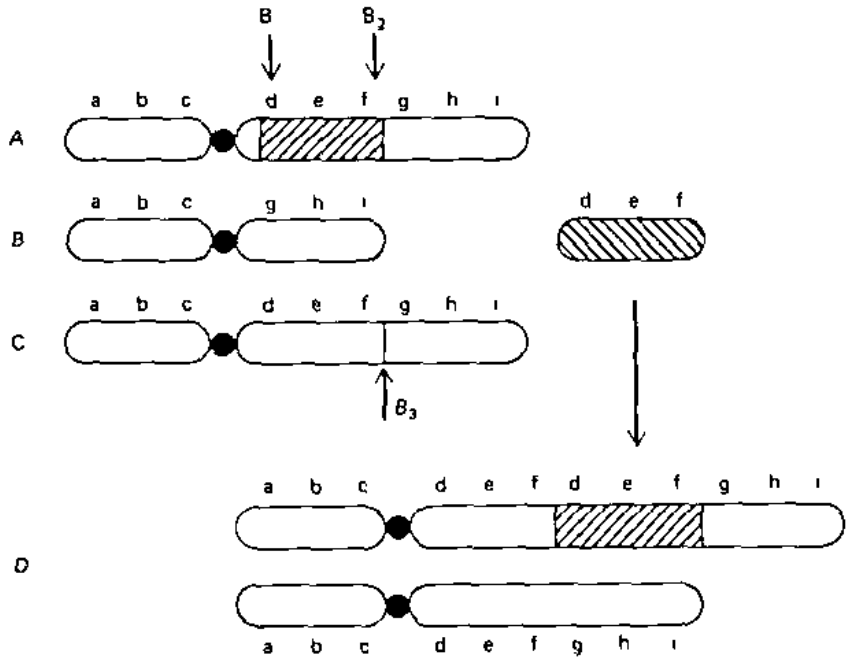


Fig 12 3A–D Diagram of a tandem chromosome duplication (*A*) The first two break points (Br_1 and Br_2) in the normal chromosome result in (*B*) a deleted centric chromosome (abc ghi) (*C*) and an acentric fragment (def) If the third break occurs in a homologous chromosome (Br_3), the acentric fragment (def) could insert into the partner chromosome resulting in (*D*) a tandem duplication

12.3 Position Effect

Geneticists talk about a **position effect** when genes or chromosome segments that are placed in new chromosomal neighborhoods cause a change in the phenotype of the individual affected due to their new position Two types of position effects are recognized (Lewis, 1950)

1 **S-type of position effect**
2 **V-type of position effect**

The S-type (or stable type) of position effect was the first discovered and is the one associated with chromosome duplication This type is confined to euchromatic regions of the chromosome One of the oldest examples for the stable type is the *Bar* locus in *Drosophila* The *Bar* effect is associated with a duplication of region 16A1 to 16A6 of the X chromosome and contains five bands, two of which are doublets If this region is duplicated (*Bar*), the facets in the fly's compound eye are reduced in number If unequal crossing over (such as demonstrated in Fig 12 4) occurs between two homologous chromosomes, both having a duplicated *Bar* region, chromosomes can result that carry the 16A1 to 16A6 region in triplicate (Fig 12 5) This situation allows a comparison of four doses of 16A1 to 16A6 in two different combinations These two different combinations are called *Bar* (homozygous *Bar*) and heterozygous *Bar double* in Fig 12 6 (Morgan et al , 1935)

Bar produces an average number of 68 facets in the compound eye and heterozygous *Bar double* produces only 45 Obviously, the gene expression is stronger

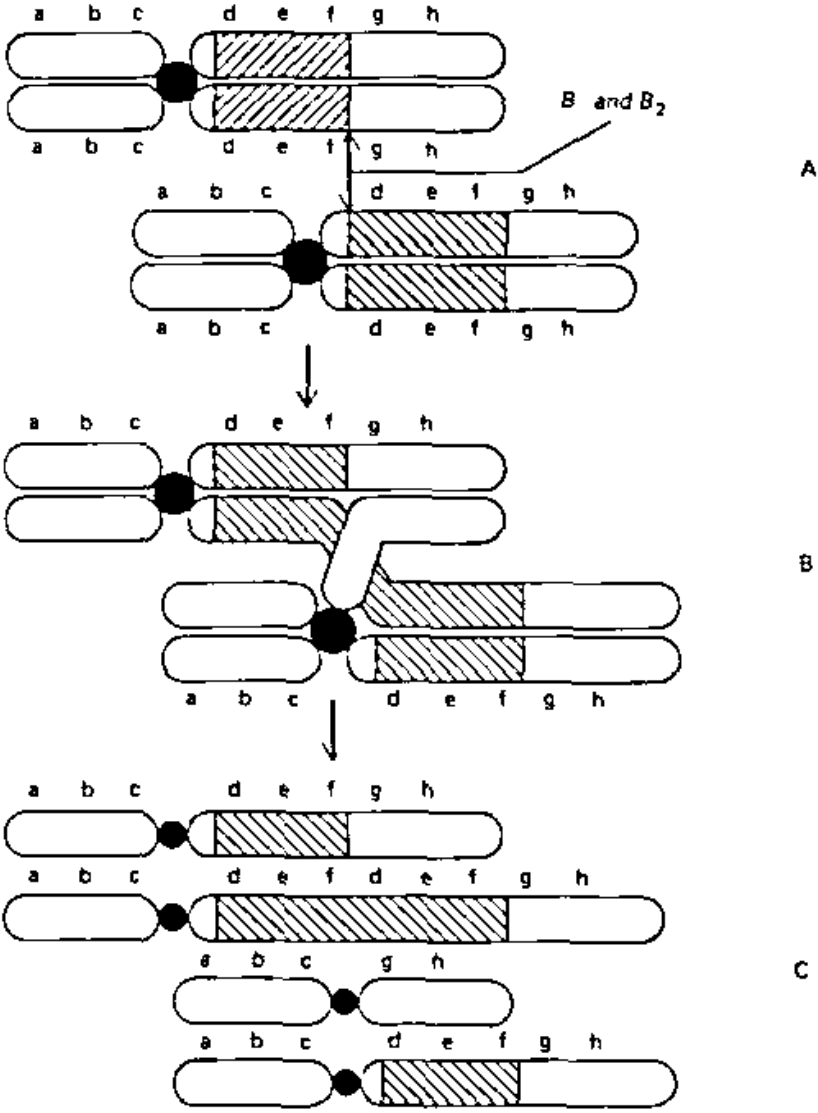


Fig 12 4A C Diagram of chromosome duplication as a result of **unequal** crossing over (*A*) Two normal homologous chromosomes with breakpo nts (Br and Br_2) in non sister chromatids (regions fg and cd) (*B*) The four chromatids after crossing over occurred at the *breakpoints* (*C*) The *resulting four chromosomes* Two chromosomes *are normal* (abc defgh) one is duplicated (abc defdefghi) and one is deleted (abc ghi)

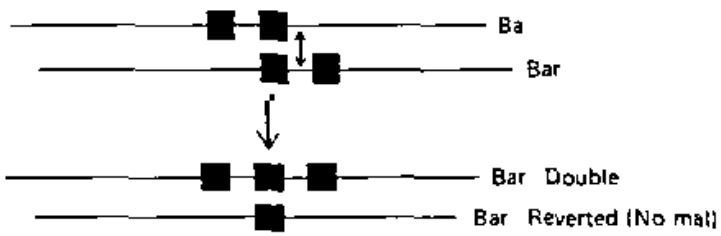


Fig 12 5 Origin of *Bar-double* by unequal crossing over in the *Bar* locus of the salivary gland X chromosome of *Drosophila melanogaster* (From Morgan et al 1935 Redrawn by permission of the Carnegie Institution of Washington Cold Spring Harbor New York)

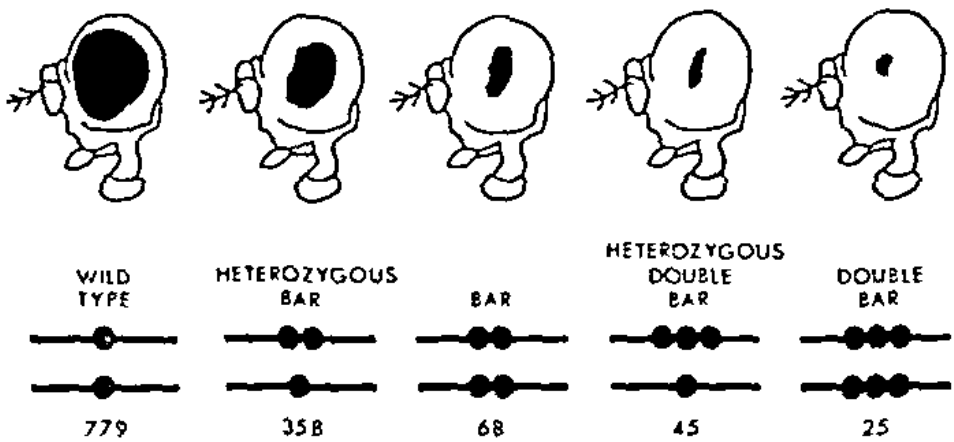


Fig 12 6 Illustration of the different sizes of compound eyes of the female *Drosophila melanogaster* as caused by the varying numbers of facets The size of the eye is influenced by the position effect (From King 1965 Redrawn by permission of Oxford University Press New York)

when the genes are adjacent in the same chromosome than when they are on separate homologous chromosomes

The V type (or variegated type) of position effect was closely associated with the production of reverse tandem duplications observed in maize by McClintock in 1951 (Section 12 1) This type is limited to genes that are present in the heterozygous state and results in heterochromatinization and repression of a wild-type gene if this gene is transferred into the vicinity of heterochromatic chromosome segments In contrast to the S-type, the V-type is subject to a large degree of fluctuation The phenotype expresses a mixture of cell patches of both the wild type and the recessive phenotype The result is a somatic mosaicism that Schultz (1936) called **variegation.** This type of position effect is often associated with the translocation and inversion type of chromosome aberration and will be discussed further in the following chapters The classical example for the V-type position effect is the **Activator-Dissociation system** (*Ac-Ds*)

12 3 1 The *Ac-Ds* System

This system was discovered by McClintock (1950a, 1950b, 1951, 1953,) in maize and depends on the action of two separate loci, the *Ac* locus (**Activator**) and the *Ds* locus (**Dissociation**). *Ds* only functions in the presence of *Ac* If both loci are present, chromosome breakage is increased in the organism Breakage has led to such chromosome aberrations as deficiencies, duplications, inversions, translocations, and ring-chromosomes

Ac and *Ds* are visualized as blocks of heterochromatin that can move to different sites of the chromosome complement This phenomenon was called **transposition** by McClintock *Ds* was discovered first and was close to the locus wx on chromosome 9 Other locations of *Ds* were discovered later No standard position was found for *Ac* Since *Ac* does not have a mutating effect on neighboring genes as *Ds*, it is more difficult to map *Ac* However, *Ac* is also capable of transposition

Not only the location of *Ac* and *Ds* in the chromosome complement but possibly also the amount of heterochromatin involved can change Such a dosage effect could be particularly well studied in triploid endosperm of maize where from 0 to 6 dosage factors of *Ac* could be observed The larger the number of *Ac* dosage factors were the later *Ds* was expressed during endosperm development As a matter of fact in kernels where four *Ac* dosage factors were present, the time of *Ds* chromosome breaks was so much delayed that none occurred before the endosperm growth had been completed The earlier during development the *Ds* locus became active the larger were the patches of variegated tissue in the endosperm
Systems similar to the *Ac-Ds* system have been observed in maize, *Drosophila* and bacteria An example of the variegated type of position effect in *Drosophila* is the variegated eye color gene in the X chromosome (Glass, 1933, Baker, 1963)
The similarity between the maize and bacterial systems was emphasized by McClintock (1961, 1965) and Peterson (1970) McClintock adapted the terms used in bacteria to the maize system The action of the **structural gene** (e g , *wx* in maize) may come under the control of a foreign element (*Ds*) at the gene locus that would be comparable to an **operator gene.**
The control of time and frequency of occurrence of change in action of the structural gene is determined by the *Ac* **regulator gene** (Jacob and Monod, 1961a and b, Chapter 1)

12.4 Other Phenotypic Effects

The phenotypic expression of duplications generally is not as strong as that of deficiencies Few duplications have unique phenotypic effects As is the case with deficiencies in plants the gametophyte is more easily affected by a duplication than the sporophyte As described in the case of the *Bar* locus in *Drosophila* some duplications not only increase the genetic effect of a gene, but they actually behave like dominant mutations Several of the duplicated chromosome segments behave like dominant mutations in the heterozygous condition Examples are the *Theta Pale* and *eyeless-Dominant* duplications in *Drosophila* The *Theta* (*Th*) duplication was discovered by Muller and Painter (1929) and involves the duplication of the left end of the X chromosome including the loci for *y sc* and *bb* The duplicated segment is attached to the centromere region at the right end of the X chromosome (Bridges and Brehme 1944) This duplication causes the development of interalar (between the wings) bristles that are not ordinarily present in *Drosophila melanogaster*
The *Pale* (*P*) character is a result of an interchromosomal or displaced duplication such as shown in Fig 12 1C It involves a transposition of a chromosome 2 segment into an interstitial position of chromosome 3 It is the result of an aneuploid segregant from a T(2,3)P translocation (Morgan et al , 1935) This was the first discovery of a translocation in *Drosophila melanogaster* The phenotypic expression of this heterozygous duplication is a dilution of the eosin eye color
The *eyeless Dominant* character (ey^D) also discovered by Muller (Patterson and Muller, 1930) involves an unidentified segment transposed into an interstitial posi

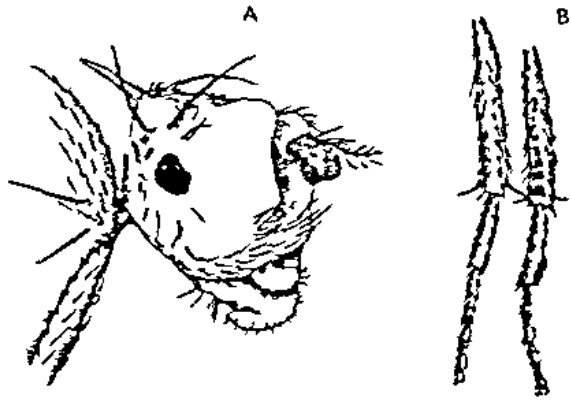


Fig 12.7A and B Drawing of the eyeless dominant character (ey^D) in *Drosophila* caused by an unidentified segment transposed into an interstitial position in the middle of chromosome 4 (*A*) head (*B*) first pair of legs (From Patterson and Muller 1930 Redrawn by permission of the Genetics Society of America Austin Texas)

tion in the middle of chromosome 4 (Sturtevant, 1936) It is suspected that the transposed segment is a reversed repeat since it forms a buckle bending back on itself in synapsis The phenotypic expression of this heterozygous duplication includes small, irregularly outlined eyes that are displaced toward the top rear of the head (Fig 12 7) The homozygous condition produces complete lethality during the larval period

12.5 Human Chromosome Duplication Syndromes

Chromosome duplications per se in humans had not been discovered until recently They have been demonstrated in connection with pericentric inversion progenies They are designated as duplication-deletion syndromes (Stevens, 1974, Welch, 1974, Allderdice et al , 1975) The pericentric inversion, which is the progenitor of

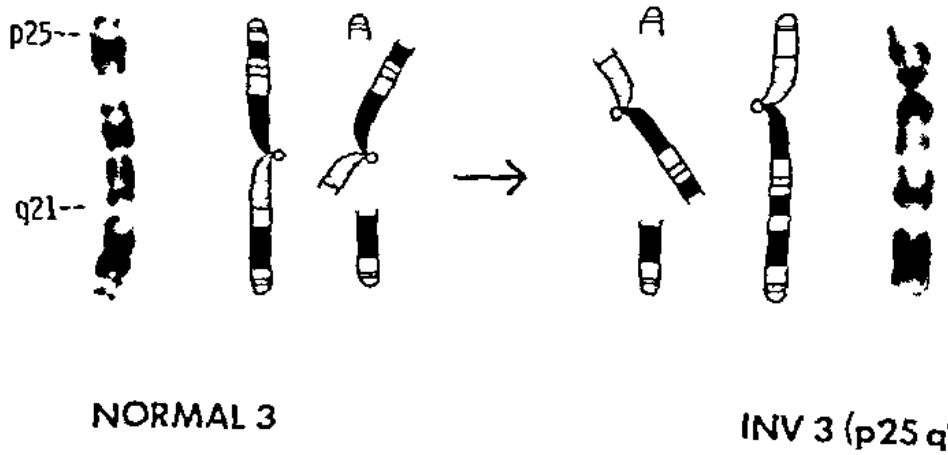


Fig 12.8 Diagram and actual photographs of pericentric inversion in human chromosome 3 showing postulated two breaks (p25 and q21) in a normal chromosome 3 (From Allderdice et al , 1975 Reprinted by permission of the University of Chicago Press, Chicago)

Chapter 13
Chromosome Inversions

Inversions are probably the most common type of chromosome aberrations found in natural animal and plant populations (Darlington, 1937 Dobzhansky 1941) During the discussion of chromosome duplications, we saw that chromosome segments can be separated from their mother chromosome by breaks and then can be reinserted into another homologous chromosome in the reverse order (reverse tandem duplications, Chapter 12) If no duplication is involved in such a process, the chromosome aberration is called an ***inversion.*** Just as in the case of deletions and duplications, organisms can be heterozygous for an inversion, homozygous for an inversion, or homozygous for the standard order of genes in the chromosome (Fig 13 1)

Chromosome inversions have no effect on mitotic divisions, but they do affect meiosis If an inversion is in the heterozygous condition, pairing of chromosomes cannot occur in a simple linear fashion But if the inverted chromosome segment has the proper size, a loop can form that satisfies the pairing requirements Depending on the occurrence of the inversion in relation to the centromere, two different kinds of chromosome inversions are known (1) pericentric inversion and (2) paracentric inversion In the pericentric type of inversion, two chromosome breaks occur one on each side of the centromere, involving both chromosome arms In the paracentric type, both breaks occur in the same arm (Fig 13 2) The paracentric type of inversion is more common in submetacentric and subtelocentric chromosomes

13.1 Pericentric Inversions

In a **pericentric inversion,** the centromere is included in the inverted region This usually results in a morphological change of the chromosome due to change in centromere position and arm ratio This kind of chromosome aberration can easily be detected in the karyotype (Fig 13 2) Depending on the size of the inverted chromosome segment, different meiotic pairing configurations may be formed in the **inversion heterozygote.** If the inverted region is small, the homologous chromosomes may not pair in that particular region of the chromosome (Fig 13 3A) If the inverted region is large enough for maneuvering, the two relatively inverted segments of two homologous chromosomes in an inversion heterozygote can pair

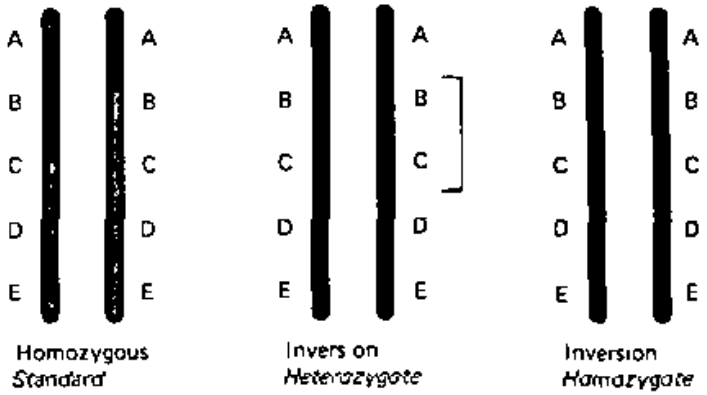


Fig 13 1 A pair of chromosomes homozygous for the standard order of genes, heterozygous for an inversion, and homozygous for that same inversion (Adapted from Srb et al 1965)

gene by gene by forming an **inversion loop** (Fig 13 3B) If the inverted region includes the major length of the chromosome, only the relatively inverted region may pair while the uninverted chromosome ends outside the inversion may remain unpaired (Fig 13 3C)

In instances where the inverted regions in the inversion heterozygote are extremely small (Fig 13 3A), no further changes would be encountered during meiosis But if the inverted region is extremely large (Fig 13 3C), crossing over in the inverted segment can result in unbalanced recombinant chromosomes In the case of inversion loop formation crossing over inside the loop will lead to further complications One crossover will produce deficiency-duplication chromatids An example of such development is shown in Fig 13 4 This figure demonstrates the meiotic consequences of a pericentric inversion in chromosome 3 of humans as discussed in Chapter 12 (Allderdice et al , 1975) In this instance the crossover must have

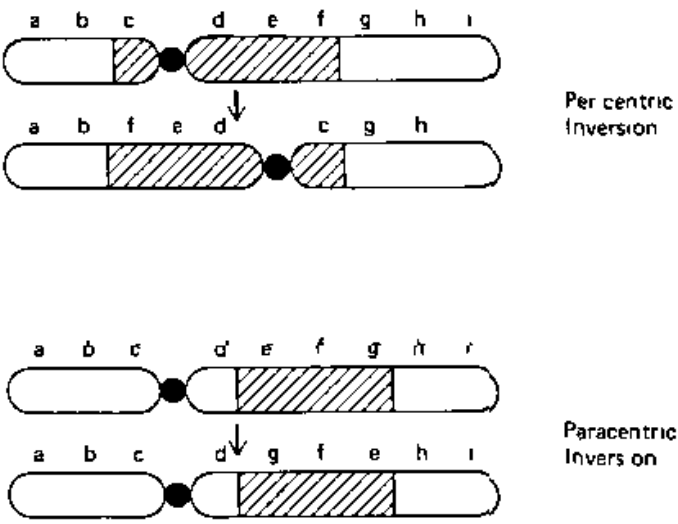


Fig 13 2 Drawing of pericentric and paracentric inversions In a pericentric inversion, the centromere is included in the inverted region In a paracentric inversion the centromere is not included in the inverted region The paracentric inversion is more common in submetacentric and subtelocentric chromosomes

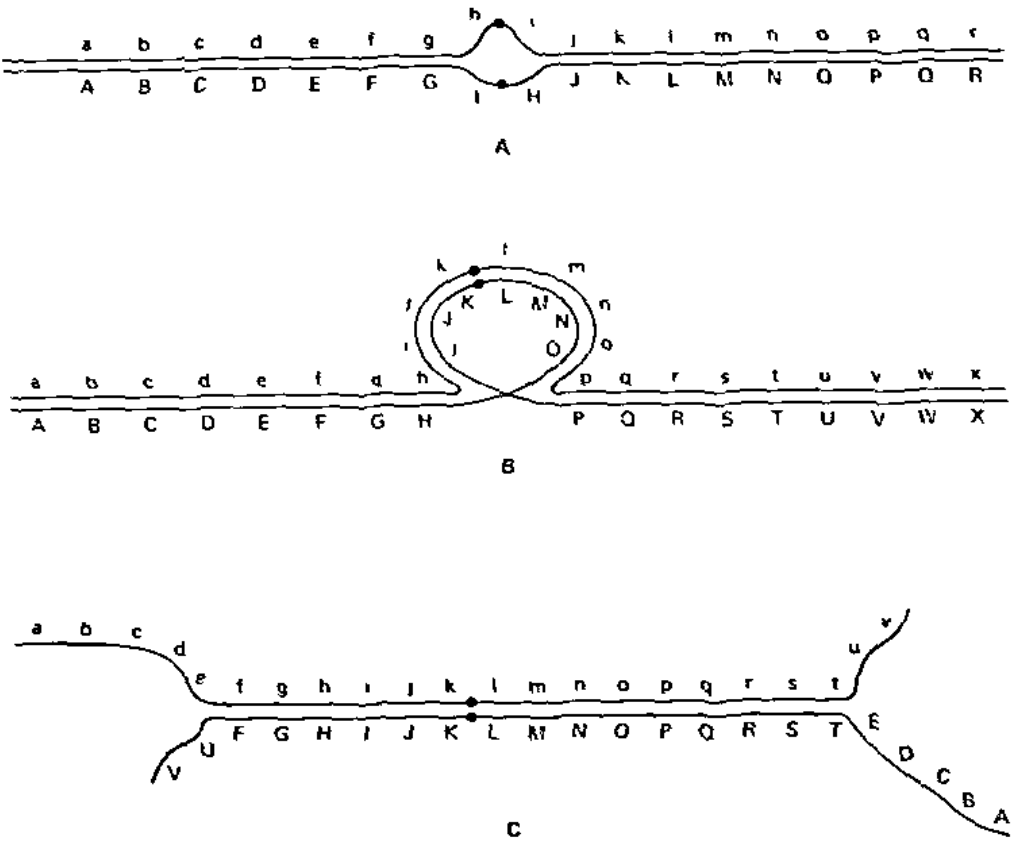


Fig 13 3A–C A drawing of inversion heterozygote chromosomes when paired in meiosis. (*A*) Example of chromosome pairing when the inverted material is very small in content (*B*) *Example of the inversion loop. This condition occurs when the* inverted region of the chromosome is large enough for maneuvering One chromosome buckles while its heterozygote partner loops, and then these chromosomes pair gene for gene (*C*) Example of an inversion that includes the major length of the chromosome The majority of the chromosome pairs in the inverted region leaving the uninverted ends of the heterozygote chromosomes to dangle

occurred fairly close to the centromere (between D and E Letters are used arbitrarily in Fig 13 4B) The four recombinant chromosomes resulting from such a crossover are shown in Fig 13 4C Two of these involve duplication-deficiencies One of them shows a larger duplicated segment (ghi), and it lacks a small segment (a) This chromosome (shown in the square) has the constitution ihgbcd EFGHI The other recombinant chromosome has a smaller duplicated segment (a) and lacks a larger segment (ghi) The chromosome with the *smaller deficient segment* has the better chance to survive since deficiencies are more likely to be detrimental than duplications Indeed, the autosomal monosomic condition for the large deficiency has not been identified in leucocyte tissue culture The recombinant with *the q duplication and the p deletion* (*Fig* 13 4) has been identified by banding patterns in karyotypes from one fetus and four children of known ins (3) (p25q21) carriers (Allderdice et al , 1975) The deleted segment reaches from the end of

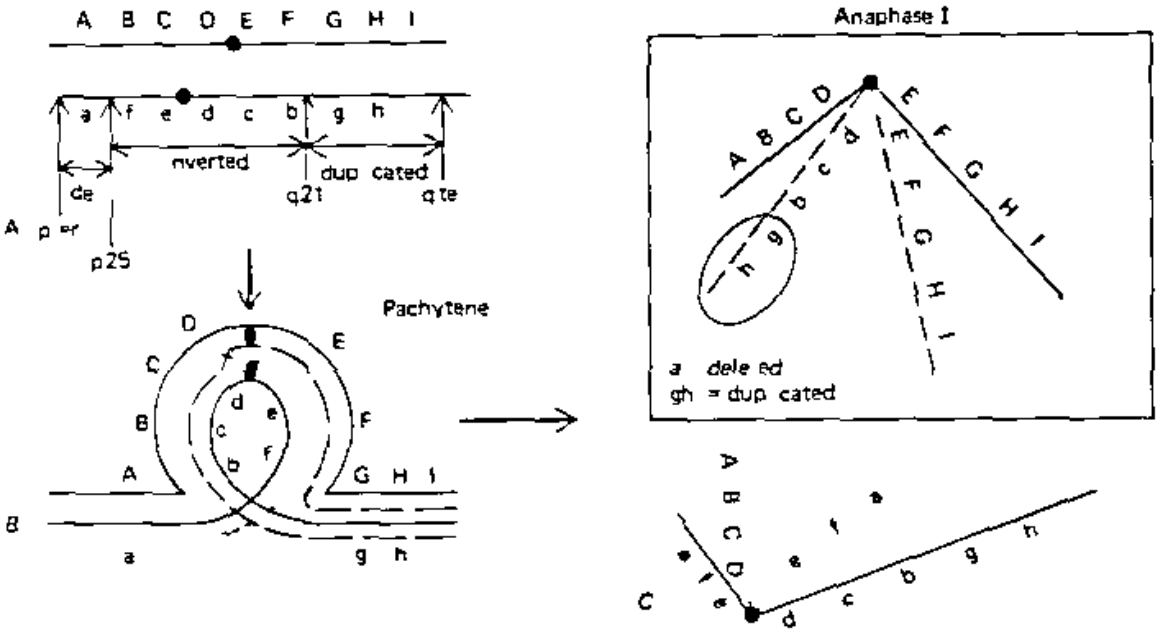


Fig 13 4A C Pericentric inversion in human chromosome 3 (p25q21) (*A*) Illustration of the two chromosomes of an inversion heterozygote (capital letters normal chromosome small letters inversion chromosome) (*B*) Meiotic pairing configuration in inversion heterozygote with crossing over in inversion loop (dE) close to the centromere (*C*) The four recombinant chromosomes resulting from a crossover One of the chromosomes in the square shows a larger duplicated segment (ghi) and a lacking small segment (a)

the short arm (pter) to band p25 The duplication reaches from the end of the long arm (qter) to band q21 (see Fig 13 4A)

Another case of a family with presumptive pericentric inversion heterozygosity of chromosome 2 in humans was reported by De Grouchy et al (1966) Figure 13 5 shows the unfortunate reproductive history of the maternal grandmother of this family she showed normal intelligence but had two spontaneous abortions and a pair of stillborn twins Here as in the previous case described by Allderdice et al (1975) the most likely cause of chromosome imbalance in this family was suspected to be crossing over within the inversion loop which results in a chromosome carrying a duplication-deficiency

Instances of extremely small pericentric inversions in humans were recorded by de la Chapelle et al (1974) They reported such an inversion in chromosome 9 (p1q13) of 35 individuals that were related to each other Two similar pericentric inversions in chromosome 10 (p11q21 and p11q11) were detected by them in two sibs of 11 and 8 individuals each They concluded that minor pericentric inversions are readily propagated and do not lead to mitotic or meiotic disturbances They estimated the incidence of these inversion types in the Finnish population as being above 1% which exceeds previous reports

Other presumptive pericentric inversions in men have been reported by Jacobs and Ross (1966) in the Y chromosome by Gray et al (1962) and Schmid (1967) in the G group and by Court Brown (Court Brown et al 1966 Court Brown 1967) Ferguson-Smith (1967) and Jacobs et al (1968) in the C group

If two crossovers involving the same two chromatids (**two-strand double crossing**

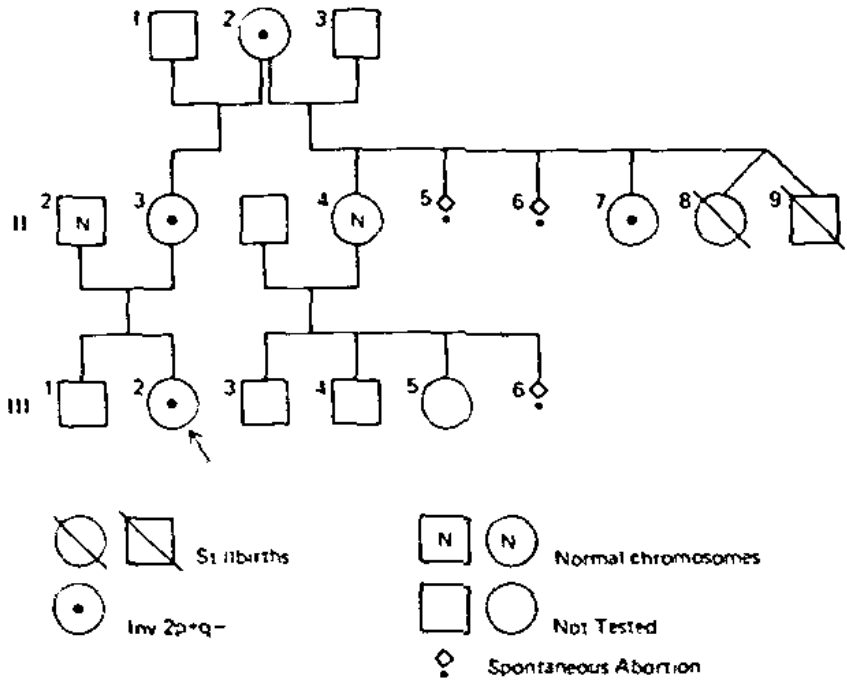


Fig 13 5 A family tree drawing of a presumptive pericentric inversion of chromosome 2 in humans Because of crossing over in an inversion loop, duplication-deficiency carrying chromosomes result (From De Grouchy et al 1966 Redrawn by permission of Academic Press, Inc , New York)

over) occur within the inversion loop, no duplication-deficiency chromatids will be formed A second crossover cancels the effect of the first one (Fig 13 6) Any odd number of crossovers within the inversion loop involving the same two chromatids (strands) will cause duplication-deficiency chromatids or gametes Any even number of such crossovers will cancel this effect If more than two chromatids are involved in such crossing over (e g , three-strand double crossing over, four-strand double crossing over) within the pericentric inversion loop, then duplications and deficiencies do occur and the effect is not canceled

As demonstrated, crossing over leads to duplications and deficiencies in the

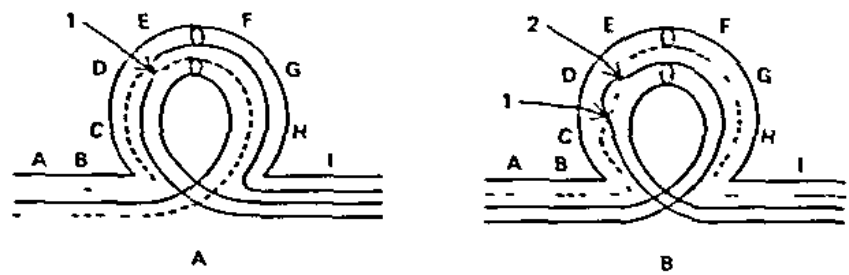


Fig 13 6 (*A*) Drawing of two-strand double crossing over when only one crossover occurs in the inversion loop Duplication-deficiency chromatids result (*B*) If two crossovers occur in the inversion loop, the second crossover cancels the cytological effect of the first crossover

gametes produced by pericentric inversion heterozygotes. Such chromosome aberrations have led to reduced gamete fertility (Alexander 1952, Patterson and Stone 1952). This may be the reason for the low frequency of observed pericentric inversions in the genus *Drosophila* as compared to the occurrence of paracentric inversions in this genus. However, small pericentric inversions are apparently much more frequent in some animal groups than previously suspected. Gene rearrangements induced by such inversions have been detected which cause polymorphic karyotypes within the species *Rattus rattus* and *R. norvegicus* (Yosida and Amano 1965, Yosida et al. 1965).

Different karyotypes originated by pericentric inversions causing chromosome polymorphism have been observed in some natural grasshopper populations (White 1948, Lewontin and White 1960, White et al., 1963). Polymorphic populations can be in a karyotype equilibrium. In such an equilibrium the frequencies of the different karyotypes do not change conspicuously from generation to generation.

Many plant genera are also known to have inversions, but their frequency is much less than in animals (Snow 1969). Only three pericentric inversions have so far been identified in fungi such as *Neurospora* (Newmeyer and Taylor 1967, Turner et al. 1969).

13.2 Paracentric Inversions

Paracentric inversions occur more frequently than pericentric inversions in natural populations. As mentioned before, in this case the centromere is not included in the inverted segment (Fig. 13.2). A photograph of a paracentric inversion heterozygote in the X chromosome of *Drosophila* is shown in Fig. 13.7. In contrast to pericentric inversion heterozygotes, paracentric ones produce **anaphase bridges** and **acentric fragments** in meiosis if crossovers occur within the inversion loop. Consequently, in natural or irradiated organisms the occurrence of anaphase bridges and acentric fragments is an indication that paracentric inversions may be present. But anaphase bridges and acentric fragments do not only occur as a consequence of this type of inversion. Other possible reasons for their occurrence could be spontaneous breakage and fusion of chromosomes during meiosis (Haga, 1953) as well as chromosome breakage and sister chromatid reunion as observed in the meiosis of rye, inbred for 27 generations (Rees and Thompson, 1955).

As discussed for pericentric inversions, the occurrence of crossing over in the inversion loop will also have an effect on the chromosomes of paracentric inversion heterozygotes. Depending on the number of crossovers within and outside the inversion loop and on the number of chromatids involved in the crossing over, anaphase bridges and acentric fragments will be single or double and can occur in anaphase I or anaphase II (Table 13.1). Only some of the possible combinations will be illustrated here for demonstration of the meiotic and genetic consequences. A master diagram illustrates the crossover points involved in these examples (Fig. 13.8). Crossover point I involves chromatids 2 and 4, crossover point II involves chromatids 1 and 4, crossover point III involves chromatids 2 and 3, and crossover point IV involves chromatids 1 and 3. Crossover points I to III are inside the inversion

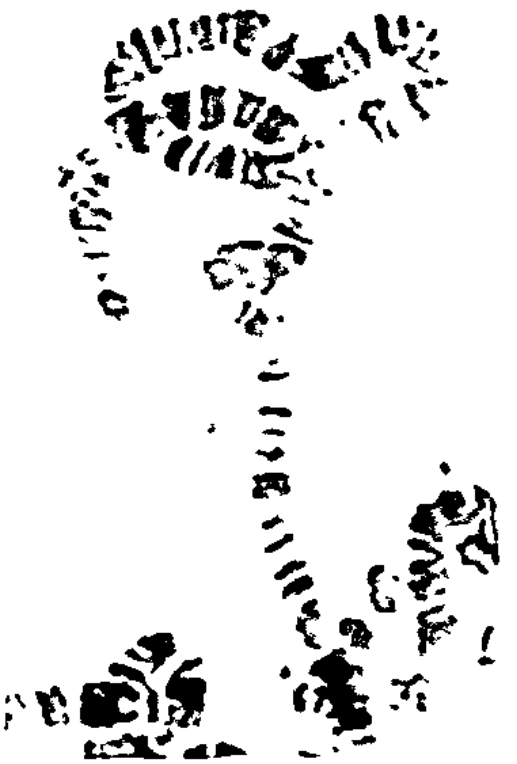


Fig 13 7 Photomicrograph of a paracentric inversion heterozygote [In (1)d1-49] in the X chromosome of *Drosophila* (Courtesy of Dr Joseph B Morton, Cargill Inc Lubbock, Texas)

loop while crossover point IV is located between the centromeres and the inversion loop If a crossover occurs only at point I (two-strand single crossing over), a bridge and an acentric fragment result in anaphase I (Fig 13 9) Since the acentric fragment does not inherit a centromere it will not be included in any daughter nucleus, and its genetic information will be lost. It may still be visible in the second meiotic division but since it is eventually abandoned it is not shown in the diagram. The size of the acentric fragment gives an indication of the size of the chromosome segment that is inverted. The acentric fragment represents the length of the

Table 13 1 Inversion heterozygotes with different types of crossing over and consequences in anaphase I and II

Strands	Crossing over	C-I*	I †	AI	AII
2	single	X	—	normal	normal
2	single	—	X	1B + 1F	normal
4	double	—	XX	2B + 2F	normal
3	double	—	XX	1B + 1F	normal
3	double	X	X	1F	1B
4	triple	X	XX	2F	2B

*C I = crossover between centromere and inversion region
† I = crossover within inversion region
B = bridge
F = fragment

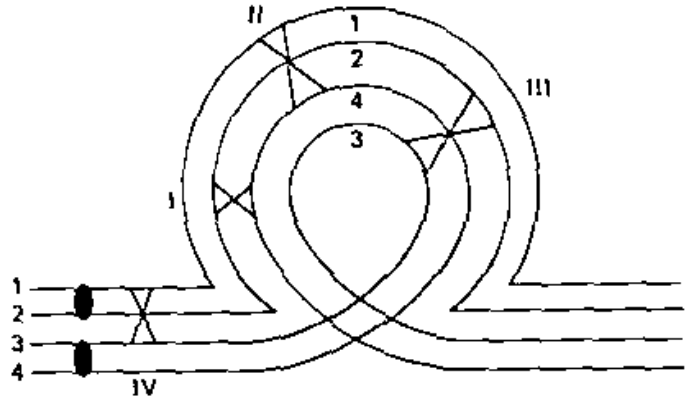


Fig 13 8 Master diagram for inversion loop crossovers Crossover point I involves chromatids 2 and 4 point II involves chromatids 1 and 4 point III involves chromatids 2 and 3 and crossover point IV involves chromatids 1 and 3

inverted region plus twice the length of the uninverted region from the distal break point to the end of the chromosome In Fig 13 9 the four differently indicated lines show the four chromatids in pachytene after crossing over has occurred Each of the four crossover products is indicated with a uniform but different type of line These were originally supposed to be drawn in color but the expense of reproduction prohibited this procedure The student is encouraged to draw in his own colors This procedure is in contrast to other approaches that use different colors for the same chromosome to indicate the genetic changes One could call our procedure **cytological coloring** The genetic changes are indicated by the use of letters (**genetic lettering**) The two sister chromatids of the upper chromosome have been designated with small letters (a to i) and the two sister chromatids of the lower chromosome have been marked with capital letters (A to I) The crossover point in the AI (anaphase I) drawing of Fig 13 9 is indicated where the lettering changes from small letters to capital letters and vice versa (point cD in the dicentric chromosome indicated by the solid line and point Cd in the acentric chromosome indicated by the dot dash line) The dicentric chromosome is deficient for segment HI and duplicated for segment AB Bridges in AI can break at any point between the two centromeres of the dicentric chromosome (solid line) In Fig 13 9 breakage (*BP*) is assumed between E and F The break products of AI are two highly deficient monocentric chromosomes that are shown in the AII (anaphase II) drawing and in the pollen (solid line chromosomes) Gamete abortion can result from two such deficiencies Two gametes are fertile in this case As mentioned for inversion heterozygosity of the pericentric type two reciprocal chiasmata within the inversion loop which involve the same two chromatids (two-strand double crossing over) cancel each other s effect (Fig 13 6) Complementary chiasmata in the inversion loop such as in four strand double crossing over result in a double chromatid bridge and two acentric fragments in AI (Fig 13 10 Table 13 1) As a consequence all four pollen will have deficient chromosomes resulting in possible pollen sterility If this condition (four strand double crossing over within the inversion loop) is combined with two-strand single crossing over in the region between the centromere and the inversion loop then two acentric

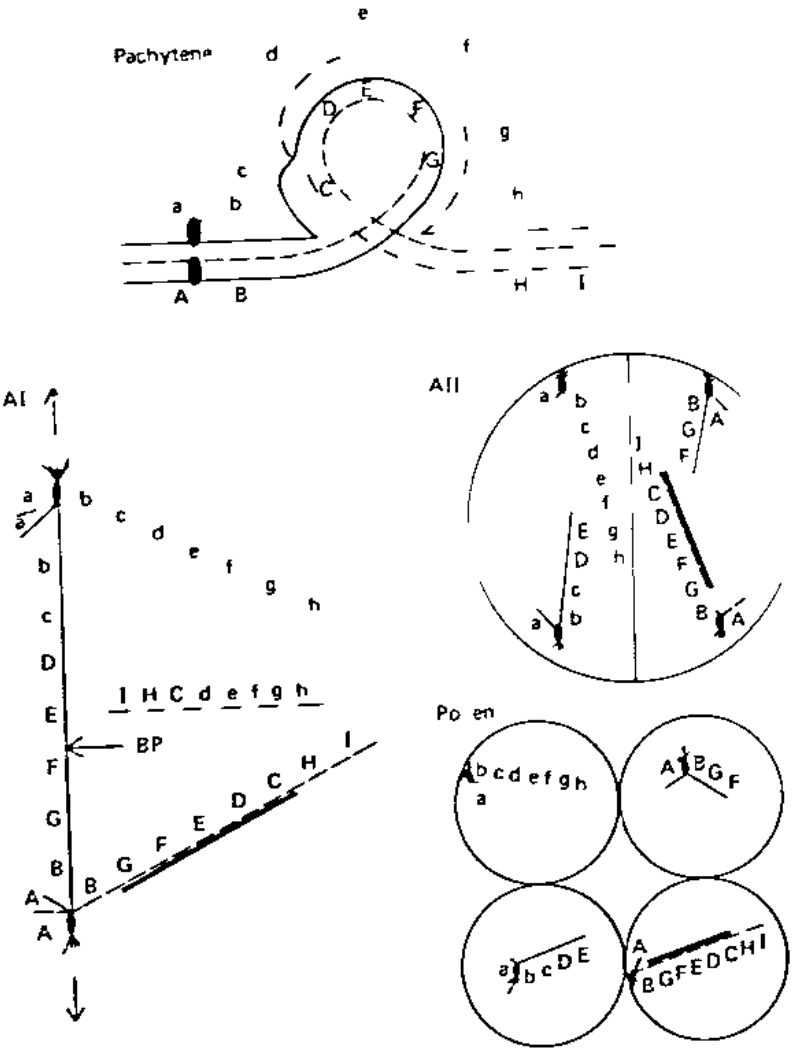


Fig 13 9 Demonstration of cytological and genetic consequences of a crossover occurring at point I of Fig 13 8 (two-strand single crossing over) The first illustration shows the 4 chromatids in pachytene Each crossover product is shown with a different line The following illustrations show the events in AI AII and at the pollen stage Genetic changes are shown by the use of letters (genetic lettering). A bridge (solid line) and an acentric fragment (dot-dash line) result in AI Two pollen can abort because of deficient chromosomes (solid lines) Heavy solid line = inverted region

fragments and two looped chromosomes will result in AI (Fig 13 11) Bridges will occur in each of the two AII cells Each of the four resulting pollen will have one deficient chromosome this will result in pollen sterility As demonstrated in Figs 13 9 to 13 11, chromatids involved in crossing over are generally eliminated by pollen or embryo sac abortion in plants or by zygote and embryo abortion in animals

In maize as well as in *Drosophila* a phenomenon has been observed that prevents the inclusion of dicentric bridges in the megaspore and the egg nucleus Carson

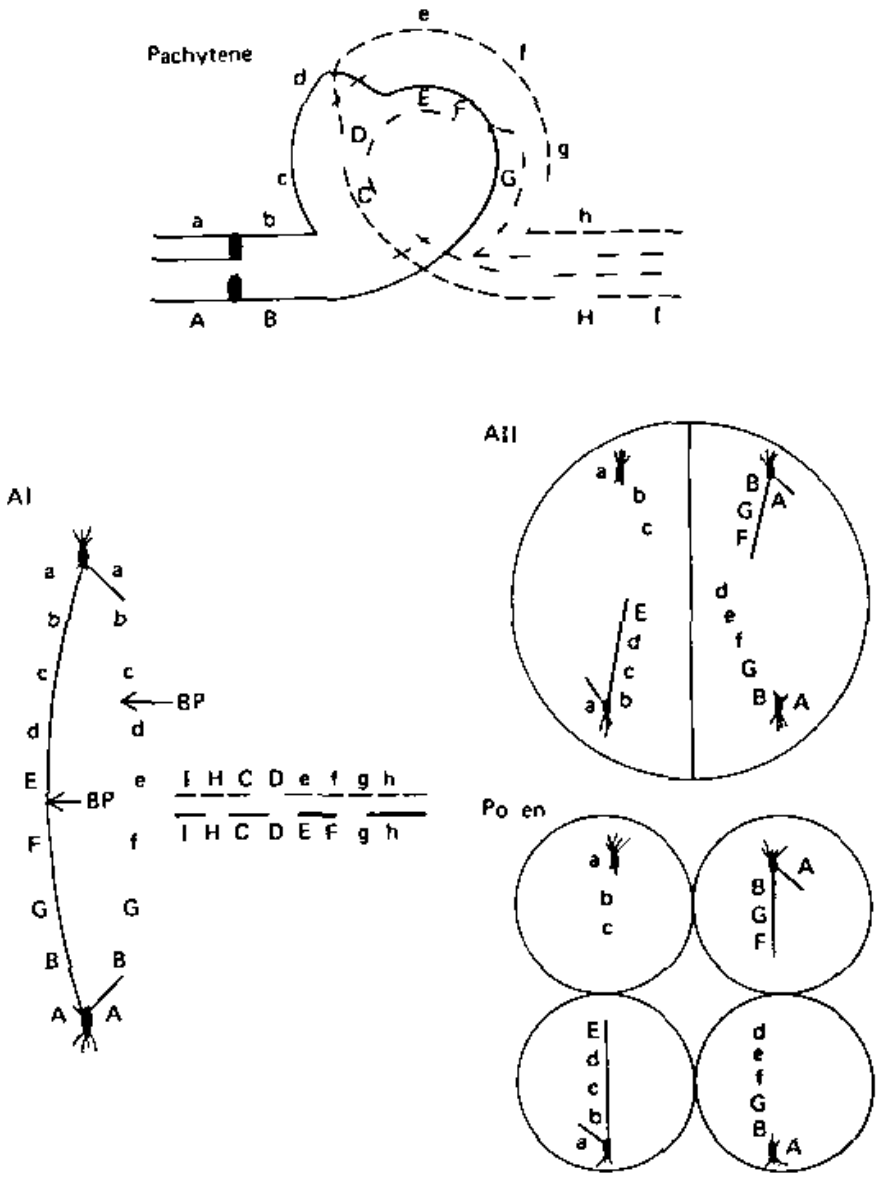


Fig 13 10 Crossovers occurring at points II and III of Fig 13 8 (four strand double crossing over) A double chromatid bridge (solid line and short-dash line) and two acentric fragments (long-dash line and dot dash line) result in AI All four pollen receive deficient chromosomes resulting in possible pollen abortion

(1946) studied more than 2500 eggs of *Sciara* He found that the dicentric chromatids formed after two-strand single crossing over in the inversion loop usually *do not break but remain as a link between the two inner nuclei (Fig 13 12)* As a result the dicentric bridge always passes into the polar bodies **(bridge elimination mechanism)** and the noncrossover chromatids are always included in the functional egg nucleus Moderate amounts of naturally occurring inversion polymorphism have been shown in mosquitoes (Kitzmiller 1976) Twenty seven different autosomal inversions have been reported from three different localities in Bulgaria in the mosquito species *Anophiles messeae* (Belcheva and Mihailova 1972)

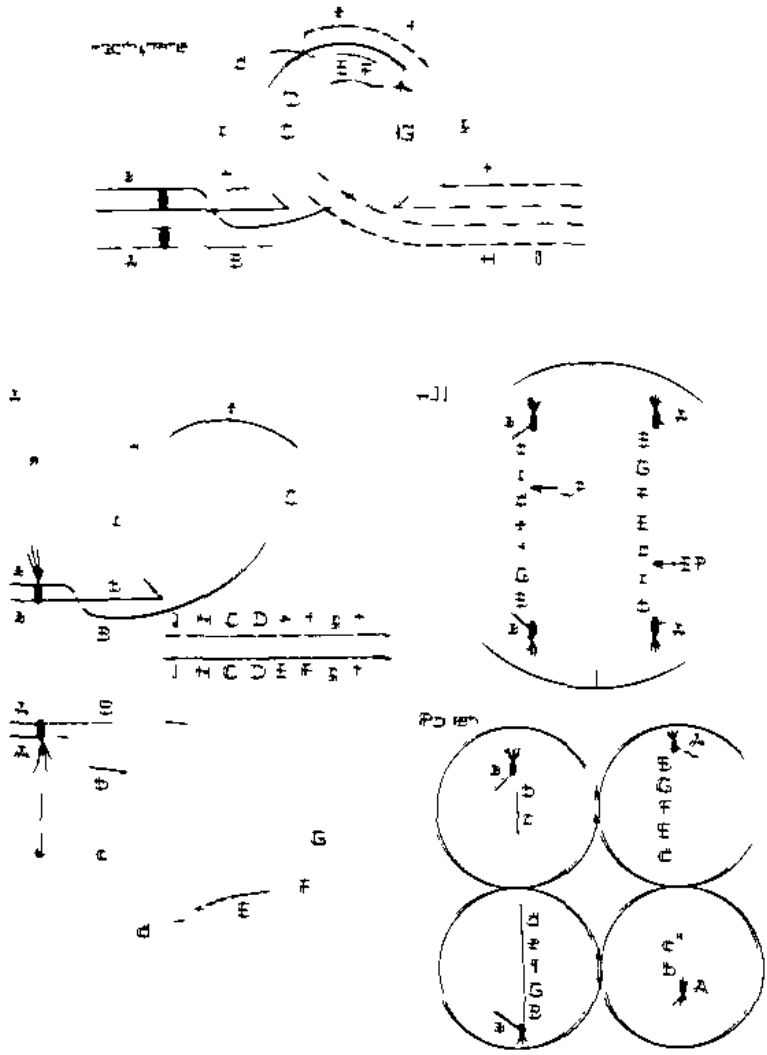


Fig. 13.11 Crossovers occurring at points II, III, and IV of Fig. 13.8 (four-strand double crossing over within the inversion region and two-strand single crossing over between the centromere and the inversion region). Two looped chromosomes (solid line and short-dash line) and two acentric fragments (long dash line and dot-dash line) result in AI. Bridges result in each of the two AII cells. All pollen will have one deficient chromosome.

13.3 Complex Inversions

If more than a single inversion is found in a chromosome, they are known as complex or multiple inversions. The types of inversions involved in a complex inversion (Rieger et al., 1976) may be:

1. independent inversion (Fig. 13.13A)
2. direct tandem inversion (Fig. 13.13B)
3. reversed tandem inversion (Fig. 13.13C)
4. included inversion (Fig. 13.13D)
5. overlapping inversion (Fig. 13.13E)

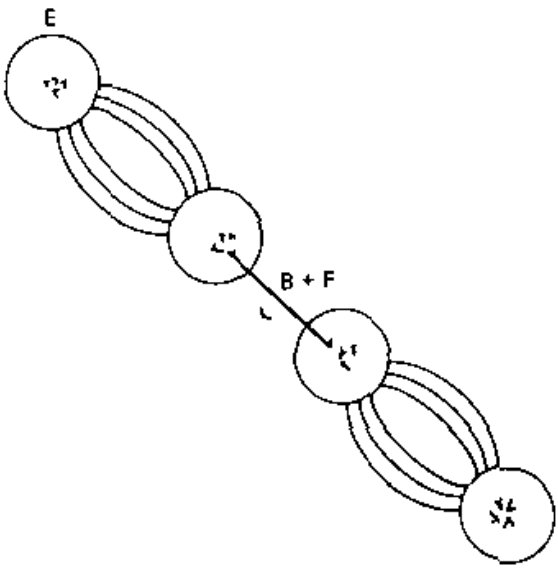
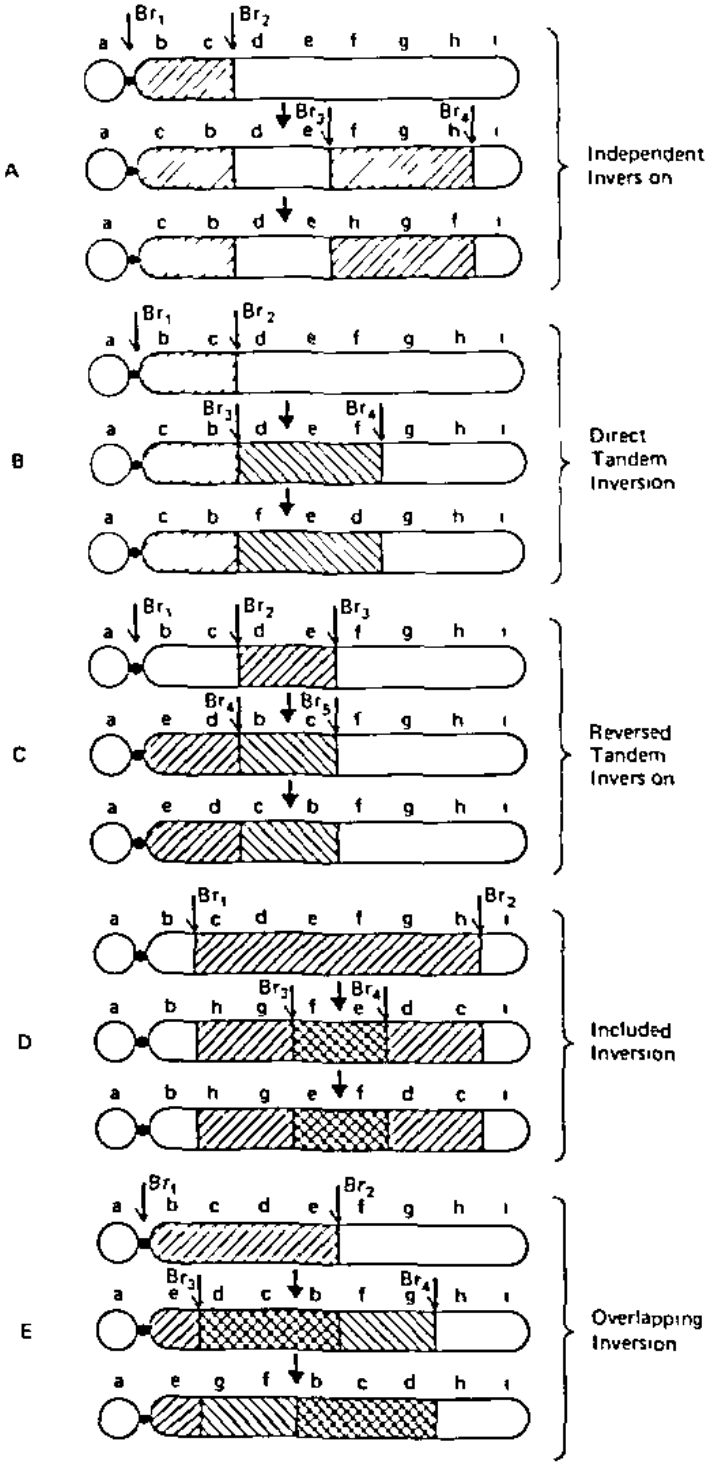


Fig 13 12 Illustration of **bridge elimination mechanism.** Decentric bridges resulting from inversions remain as a link between two inner nuclei Bridges always pass into polar bodies and noncrossover chromatids always are included in functional egg nucleus

In **independent inversions,** a chromosome inversion occurs in independent sections of the chromosome, and the two resultant inverted segments are separated from each other by an uninverted chromosome segment **Direct tandem inversions** are the result of two successive inversions involving chromosome segments that are directly adjacent to each other In **reversed tandem inversions,** the two inverted segments are adjacent to each other but mutually interchanged In an **included inversion,** a segment that is part of an inverted segment is inverted once again An **overlapping inversion** is the result of part of an inverted chromosome segment being inverted a second time together with an adjacent segment that was not included in the first inversion segment

Many complex inversions have been discovered in wild populations of *Drosophila* Over 40 different inversions were found in various chromosomes of *D willistoni* which demonstrates a great degree of **chromosome polymorphism.** Several

Fig 13 13 (A) Diagram of an independent inversion The top chromosome shows the first inversion region (bc Br_1 to Br_2) the middle chromosome shows the second inversion region (fgh Br_3 to Br_4) and the bottom chromosome shows the final condition after both inversions have occurred (B) Diagram of a direct tandem inversion The top chromosome shows the first inversion region (bc Br_1 to Br_2), the middle chromosome shows the second adjacent inversion region (def Br_3 to Br_4), and the bottom chromosome shows the resultant chromosome after both inversions have occurred (C) Diagram of a reversed tandem inversion The top chromosome shows the first inversion region (de Br_2 to Br_3) and an additional breakpoint (Br_1), the middle chromosome shows the second adjacent inversion region (bc Br_4 to Br_5), and the bottom chromosome shows the final condition (D) Diagram of an included inversion The top chromosome shows the first inversion region (cdefgh Br_1 to Br_2) the middle chromosome shows the included inversion region (fe Br_3 to Br_4) and the bottom chromosome shows the condition after both inversions have occurred (E) Diagram of an overlapping inversion The top chromosome shows the region of the first inversion (bcde Br_1 to Br_2) the middle chromosome shows the overlapping second inversion region (dcbfg Br_3 to Br_4), and the bottom chromosome depicts the final condition


A
Br1
Br2
Br3
Br4
a b c d e f g h
a c b d e f g h
a c b d e h g f
Independent
Invers on
B
a b c d e f g h
a c b d e f g h
a c b f e d g h
Direct
Tandem
Inversion
C
Br5
a b c d e f g h
a e d b c f g h
a e d c b f g h
Reversed
Tandem
Invers on
D
a b c d e f g h
a b h g f e d c
a b h g e f d c
Included
Inversion
E
a b c d e f g h
a e d c b f g h
a e g f b c d h
Overlapping
Inversion

inverted chromosome types can occur within one and the same fly population In *D pseudoobscura* and *D persimilis* chromosome inversions are also very frequent They occur mainly in the third but also in the X chromosome A female in *D willistoni* was discovered that was heterozygous for 16 different inversions Studies in *D pseudoobscura* were carried out by Dobzhansky (1941), Koller (1936), Strickberger and Wills (1966), Pavlovsky and Dobzhansky (1966), and Crumpacker and Salceda (1968)

There are two reasons why inversion heterozygosity does not result in a high degree of sterility in *Drosophila* In the males there is an **achiasmatic mechanism** and in the females a **bridge elimination mechanism.** In the achiasmatic mechanism the chromosomes are meiotically paired but no synapsis and crossing over exist Consequently, no inversion loops are formed in pachytene, and bridges and fragments do not occur in anaphase I or II, thus there is no gamete abortion The bridge elimination mechanism has already been described (Fig 13 12)

13.4 Inversions as Crossover Suppressors

A crossover suppressor can be a structural chromosome change that suppresses or reduces the frequency of meiotic crossing over The best known examples for such effects are inversion heterozygotes There are two different factors that reduce the occurrence of crossing over in inversion heterozygotes

1 Crossing over inside and around the inverted segment is reduced as a result of incomplete pairing The most drastic case is the example of a very short inversion segment that eliminates pairing altogether (Fig 13 3A) But also in cases in which the segment is large enough so that a loop is formed, crossing over can be reduced inside and around the loop as a consequence of incomplete pairing
2 The products of crossing over in an inversion loop are mostly inviable and are not recovered (Figs 13 9 to 13 11) This makes it appear as though an inversion segment in an inversion heterozygote is completely free of crossovers

In this second instance (2), the crossover frequency is inversely proportional to the length of the inverted chromosome segments Almost complete crossover suppression has been observed in short inversions of *Drosophila* species where two-strand single crossing over provides total elimination of the crossover products Incomplete crossover suppression is accompanied with longer inversions where two-strand double crossing over can occur and the second crossover cancels out the abortion effect of the first Some balanced crossover products will thus have a chance to be included in gametes

Muller (1928) was the first to make use of the crossover suppressor phenomenon in *Drosophila* He developed the *CIB* method In this method he used a special female stock that had (1) an X chromosome with a large inversion as a crossover suppressor (*C*) preventing exchange between the *CIB* chromosome and the male chromosome to be examined (black in Fig 13 14), (2) a recessive lethal gene, preventing homozygosity for the *CIB* chromosome (*l*), and (3) a *Bar* duplication, which permitted identification of individuals that carried the *CIB* chromosome in a heterozygous condition

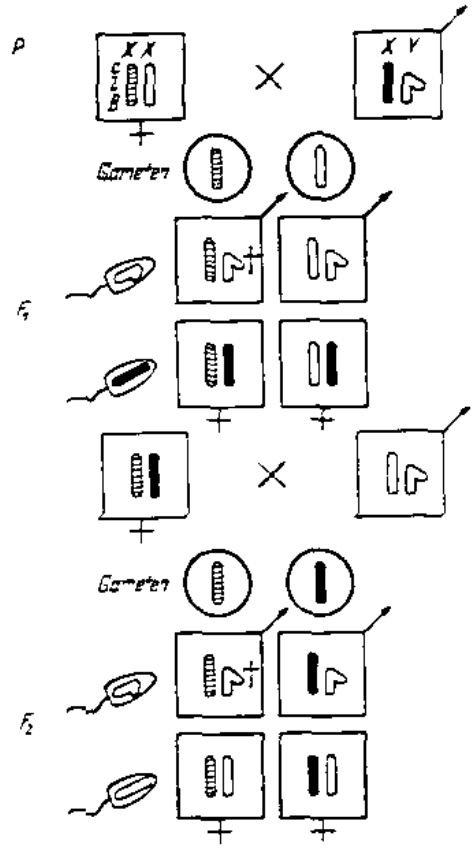


Fig. 13.14 Schematic drawing of the *ClB* method for the demonstration of recessive sex linked lethal factors in *Drosophila*. (From Rieger and Michaelis, 1958)

In this method the *ClB* females are mated with irradiated male flies (Fig. 13.14). *Half of the males in the* F_1 *generation die because they possess the ClB chromosome* in the hemizygous condition. There is no compensating *L* allele in the Y chromosome. Half of the F females are phenotypically marked (*Bar*) by the *ClB* chromosome and also possess the X chromosome of the x irradiated males. Recombination between the two X chromosomes is restricted because of the inversion. The *Bar* females are individually crossed with normal males, and each progeny is tested separately. Because all *ClB* sons of these F_2 crosses die, all surviving F_2 males possess the x rayed X chromosome from the grandfather that is to be examined (black X). If the x irradiation resulted in a mutation, all F_2 males will show the phenotypic mutant effect. If the result was a lethal mutation, no F_2 males will be observed. Because of the isolated treatment (separate test bottles) of the fertilized *ClB* F_1 females, the frequency of lethal and nonlethal mutations in the X chromosomes of the treated grandfather can be easily calculated. This method showed Muller and many other investigators the effect of specific mutagens and the resulting mutation rates.

A refinement of this tool is the M-5 method (Demerec, 1948, Spencer and Stern, 1948). This method makes use of the Muller-5 stock, which has greater crossover suppression than the *ClB* stock and does not operate with a lethal factor. The crossover suppressing inversion is of the included inversion type (Fig. 13.13D), which is a small inversion included in a larger inversion in the X chromosome. This stock

has three marker factors, the dominant *Bar* (*B*), *scute*, which is a hair factor (sc^8), and *white apricot* which is an eye color factor (w^a) The recessive markers help to identify any crossovers that may occur between the Muller-5 chromosome and the irradiated chromosome Crossover suppressors are now also developed for mutagenicity tests in mice (Evans and Phillips, 1975)

13.5 The Schultz-Redfield Effect

The presence of an inversion in the chromosome complement does not always produce only crossover suppression within the inverted segment of the heterozygote It also can have an effect on other chromosome pairs of the same complement In Chapter 4 (Section 4 3) the phenomenon of crossover interference was discussed This is based solely on a mechanical principle which states that the occurrence of a crossover in one area of a particular chromosome suppresses a crossover in an adjacent region (Whitehouse, 1965) The possibility of an interchromosomal effect of the crossover in one chromosome pair on a crossover in another such pair is automatically excluded in such mechanical consideration However, in 1919 Sturtevant found that crossing over increased between the eye color gene *purple* (*pr* 54 5) and the wing shape gene *curved* (*c* 75 5) on chromosome 2 of *Drosophila melanogaster* due to a dominant third chromosome gene In 1933 Darlington observed reciprocal effects on chiasma frequency between a B chromosome bivalent and the rest of the chromosome set in rye When the B-bivalent had high chiasma frequency, the chiasma frequency in the other bivalents was reduced and vice versa More exact evidence for such interchromosomal relationship was later given by Morgan et al (1932, 1933), Glass (1933), Komai and Takahu (1942), Steinberg and Fraser (1944), Schultz and Redfield (1951), Redfield (1955, 1957), Levine and Levine (1955) for *D melanogaster* and by MacKnight (1937) for *D pseudoobscura* In all these later cases, heterozygous inversions in one chromosome pair caused increased crossing over in other nonhomologous chromosome pairs of the same complement Schultz and Redfield in 1951 placed this effect on a quantitative basis and were credited with its discovery White and Morley (1955) speculated that the Schultz-Redfield effect could be the result of a genetic **homeostatic effect,** which keeps the crossover frequency close to an optimal value within the population, the effect being not only interchromosomal but also intrachromosomal For example, Carson (1953) demonstrated this in *D robusta* where crossing over was increased in the noninverted region of the inversion chromosome pair Rieger and Michaelis (1958) speculated that the Schultz-Redfield effect probably is not limited to the genus *Drosophila* but may be a more widely occurring phenomenon associated with heterozygous paracentric inversions

Chapter 14
Chromosome Translocations

The most common type of translocation is the reciprocal translocation Brown (1972) goes so far as to say that all translocations observed are with no known exception, reciprocal In order to survive all cells ought to have a balanced set of genes Cells with reciprocal translocations provide such conditions However since other types of translocations have been discussed in the literature they will be briefly mentioned here also

14.1 Types of Translocations

The nomenclature on chromosome translocations has not always been consistent For the sake of simplicity the terms used here are arranged according to the number of breaks occurring According to this system, four different classes of chromosome translocations can be distinguished

1 simple translocations (one break involved)
2 reciprocal translocations (two breaks involved)
3 shift type translocations (three breaks involved)
4 complex translocations (more than three breaks involved)

*The **simple translocation*** (Fig 14 1) is probably of more historical than practical value However, the discussion will soon show why it is still included here As mentioned, a simple translocation would be the result of a single break in a chromosome arm and the transfer of the acentric chromosome fragment to the end of another nonhomologous chromosome Evidence of such an event came first from Muller and Painter (1929) when they found that a group of third linkage group genes in *Drosophila*, *roughoid eye* (*ru* 0 0) to *scarlet eye* (*st* 44 0), were linked to the genes of the second linkage group The remaining genes in the third linkage group, *pink* (*p* 48 0) to *Minute-g* (*Mg* 106 2), remained independent of the genes in the second linkage group The cytological proof of this genetic observation was also given by Painter and Muller (1929)

Such an event seems to be in contrast to the earlier stated premise that telomeres seem to seal off the ends of chromosomes so that they cannot join with other broken chromosome ends (Section 2 2 4) However, what seems to be a simple translocation may in fact be a reciprocal translocation in that the very end of chromosome 2 in *Drosophila*, including the telomere, may have broken off and may have exchanged position with the acentric fragment of chromosome 3 In this way one

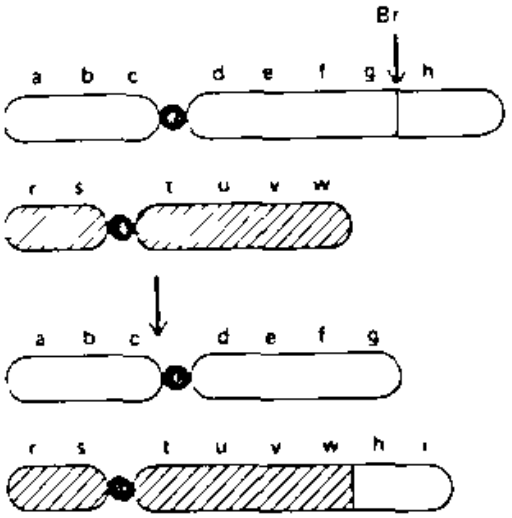


Fig 14 1 Illustration of a simple translocation The top two chromosomes are nonhomologous chromosomes with Br_1 indicating the break point on top chromosome The bottom two chromosomes are the same nonhomologous chromosomes after translocation of the acentric fragment (hi)

really deals with a two-break situation that meets the requirements of a true exchange The small fragment of chromosome 2 may not have carried any detectable genes thus simulating a condition of a simple translocation Today, it is generally believed that true simple translocations cannot occur and that all earlier reported cases are really reciprocal translocations (Burnham, 1956)

A **reciprocal translocation** involves the mutual exchange of broken chromosome fragments between nonhomologous chromosomes As mentioned, this is the main type observed and it will be discussed in great detail It is dependent on two break events (Fig 14 2)

The **shift type translocation** (Fig 14 3) or **transposition** (Section 12 3 1) depends on three breaks It can happen in three different ways

Intrachromosomal shifts

1 The broken chromosome segment can be inserted into the same chromosome arm but at a different location (Fig 14 3A)
2 The broken chromosome segment can be shifted to an intercalary position in the other arm of the same chromosome (Fig 14 3B)

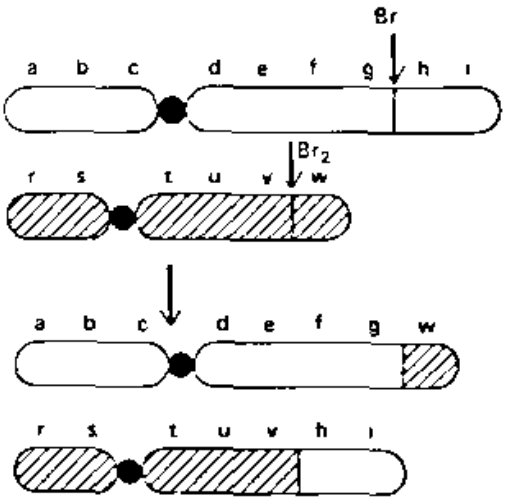


Fig 14 2 Diagram of a reciprocal translocation The top two chromosomes are nonhomologous chromosomes with break points Br_1 and Br_2. The bottom two chromosomes are the same nonhomologous chromosomes after the acentric fragments (hi, w) have translocated

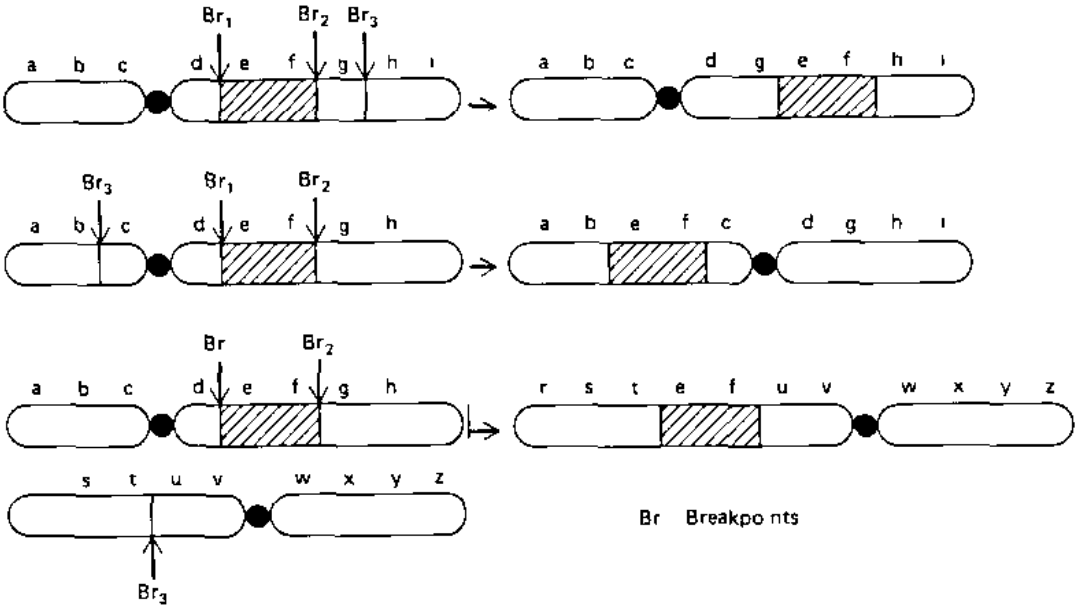


Fig 14 3A C Diagrammatic representation of the shift type translocation (transposition) (*A*) Broken chromosome segment (ef) is inserted in same chromosome arm but at different location (g↓h) (*B*) Broken chromosome segment (ef) is shifted to an intercalary position (b↓c) in other arm of same chromosome (*A* and *B* intrachromosomal shifts) (*C*) Broken chromosome segment (ef) is shifted to an intercalary position (t↓u) of a non homologous chromosome (interchromosomal shift)

Interchromosomal shifts

3 The broken chromosome segment can be shifted to an intercalary position in one of the two arms of a nonhomologous chromosome (Fig 14 3C) The first translocation found by Bridges (1923) was such an interchromosomal shift

Complex translocations are those in which three or more breaks are involved In the progeny of an irradiated *Drosophila* male, Kaufmann (1943) found an individual with a complex arrangement involving at least 32 breaks The treatment involved x rays of 4000 r followed by infrared radiation for a period of 144 hours

14.2 Origin of Translocations

Translocations can occur naturally as well as by induction As mentioned at the beginning of this discussion on variation in chromosome structure (Part VI), structural chromosome changes are generally considered to depend on breakage of chromosomes and on reunion of chromosome segments

Translocations along with other chromosome aberrations were reported by Beadle (1937) in the progeny of maize that had the gene *sticky* [*st* (55)] on chromosome 4 In such mutants the chromosomes adhere to each other in anaphase I and rupture during anaphase movement producing structural chromosome changes such as translocations A similar stickiness effect has been reported by McClintock (1950a, 1950b, 1951, 1953) in which this property is also genetically controlled by the

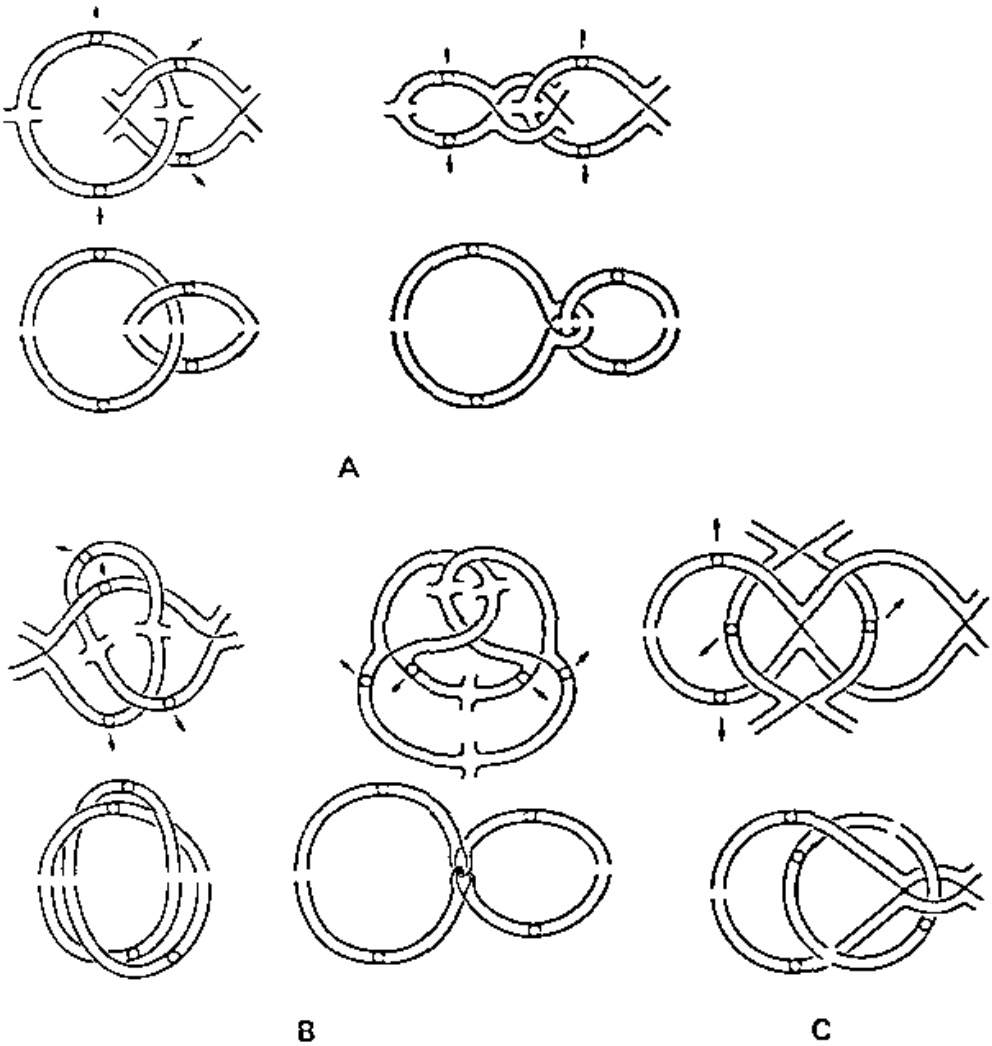


Fig 14 4A C Illustration of different types of chromosome interlocking Upper rows of *A B* and *C* diplotene Lower rows of *A B* and *C* metaphase I (Modified after Darlington 1965)

Activator Dissociation system described in Chapter 12 (Section 12 3 1) Translocations also have been found in plants that were grown from aged wheat and barley seed (Gunthardt et al 1953) Nilsson (in Kostoff 1938) in *Oenothera* and Navashin and Gerassimova (1935) in *Crepis* and other plants found that with aging seed structural chromosome changes significantly increased

Other possible causes for chromosome translocation have been reported from interlocking of bivalents that subsequently can break in anaphase I (Sax 1931 Sax and Anderson 1933 Burnham 1962) Interlocking takes place during zygotene or pachytene of first meiotic prophase when a nonhomologous chromosome passes through a loop of two homologous chromosomes that are in the process of pairing Different examples of such interlocking are shown in Fig 14 4 Interlocked bivalents are occasionally found in *Tradescantia* and in other genera in which translocation rings are found

Kostoff (1938) speculated that translocations originated from spontaneous segmental association in heterochromatic chromosome regions If Kostoff's suggestion

were true then naturally occurring translocations should show a high frequency of breakpoints in heterochromatic chromosome segments

14 3 Reciprocal Translocations

Figure 14 2 shows the mutual exchange of two chromosome segments between two chromosome pairs of nonhomologous origin If the ends of these chromosomes are numbered (1 2 3 4) then 4 chromosomes that all have different end combinations (1 2 3 4 1 4 2 3) will result No two chromosomes of this group can pair along their entire length but all four can come together in a pairing configuration (**quadruple**) that allows partial pairing of homologous chromosome segments Quadruples resemble **quadrivalents**, which are formed when all four chromosomes are homol ogous Sybenga (1972) supported the use of separate terms for these two types of configurations that resemble each other in shape but originate differently In quadrivalents all four chromosomes are homologous or equivalent to each other while in quadruples only certain segments are homologous An organism in which quad ruple pairing occurs is called a **translocation heterozygote.** In pachytene such a configuration can appear as a cross (Fig 14 5) Sometimes such a cross still can be prevalent in the following stage of diplotene (Fig 14 6) As can be seen the pairing partners in this figure change at the translocation breakpoints (Fig 14 5) Consequently, such figures can reveal the location of the breakpoints This is particularly true in instances where exact pairing occurs as in the polytene chromosomes of Diptera However, pachytene configurations in maize and tomato do not always show complete synapsis of the pairing partners involved For instance, in the heterozygous translocation strain T2-6a of maize (Burnham 1932), which has translocation breaks in the long arm of chromosome 2 and in the short arm of chromosome 6 (satellite chromosome), pairing can be gene by gene (Fig 14 7A) asynaptic or nonhomologous near the center of the cross (Fig 14 7B) Translocation breaks can occur at any point along the chromosome, possibly even in the centromere region The position of the breakpoints will determine the future fate of the translocation quadruple If the breakpoints are located close to the chromosome ends (distal area) the chance of crossover formation between the break point and the chromosome end is reduced Possible interstitial crossovers (between the centromere and the breakpoint) can lead to duplication and deficiency gametes and consequently are not recovered (Burnham, 1962) If no crossovers form in the distal area, the quadruple will break up into two open bivalents by the end of prophase I (diakinesis) and meiosis will continue mechanically normal But if crossovers are formed between the translocation breakpoints and the chromosome ends, the quadruple configurations will persist through diakinesis into metaphase I

Different kinds of quadruple configurations can arise depending on the formation of crossovers in interstitial (between breakpoint and centromere) or distal (between breakpoint and chromosome end) chromosome segments It should be remembered here that chiasmata always move away from the centromeres and not toward them (Section 7 2 4, Fig 14 5, arrows) Possible diakinesis configurations originating

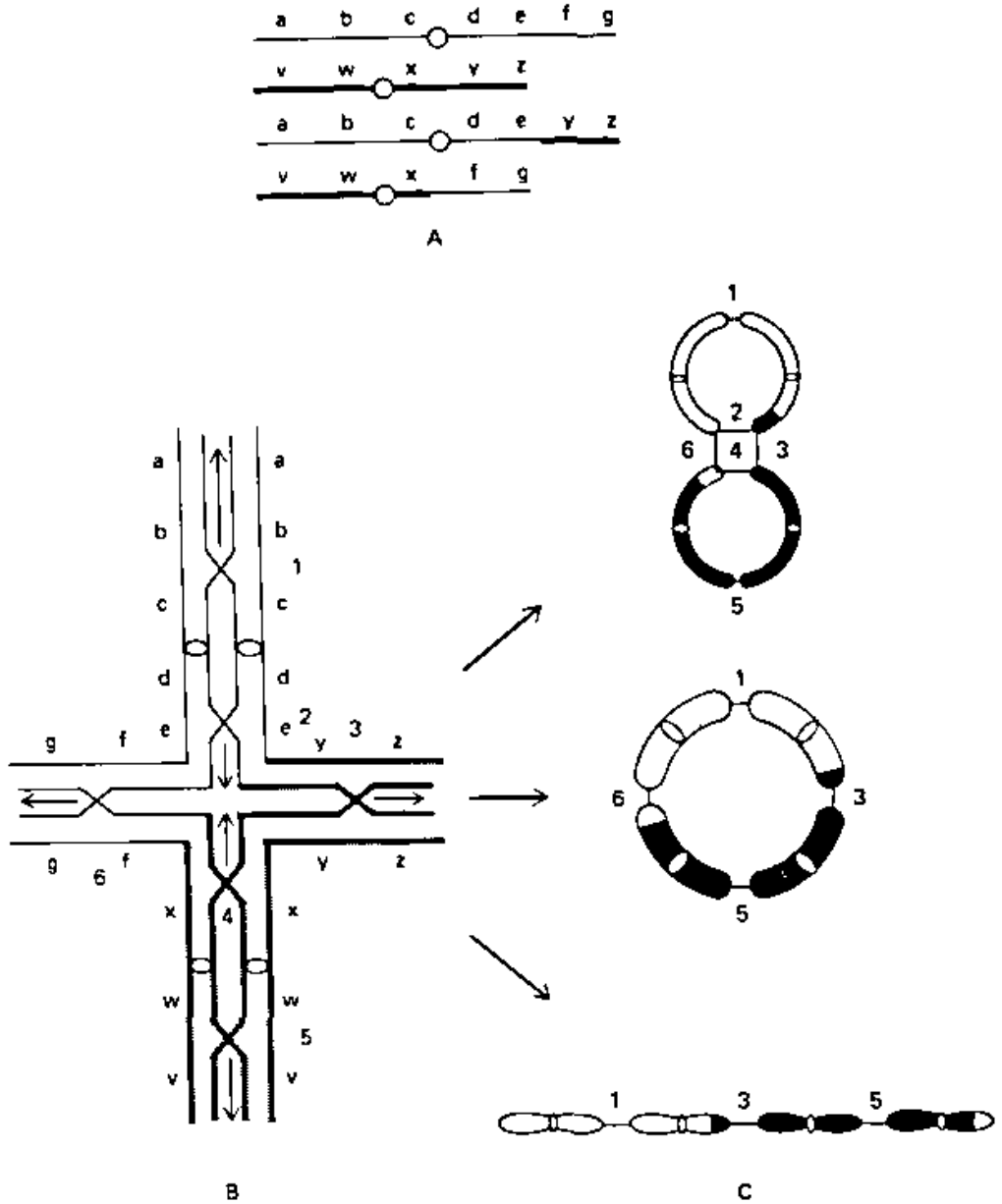


Fig 14 5A C Illustration of a quadruple pairing in a translocation heterozygote (*A*) The two homologous chromosome pairs involved in the reciprocal translocation (*B*) Pachytene configuration appearing as a cross Numbers 1 to 6 designate chiasmata (*C*) Depending on the location of the chiasmata 8 , ring , or rod shaped quadruples can form in diakinesis

from different pachytene situations are shown in Fig 14 5 If, for instance, crossovers occur in locations 1, 2, 3, 4, 5, and 6, diakinesis configurations resemble a number 8 If chiasmata occur only in locations 1, 3, 5, and 6, a ring of 4 chromosomes is formed in diakinesis If chiasmata occur in locations 1, 3, and 5, a chain of 4 chromosomes can form The crossover positions in locations 1 to 6 are minimum requirements for the diakinesis configurations indicated above Any additional crossovers in the same chromosome segments will lead to identical configurations

Chromosome orientation of quadruples in metaphase I is critical Bivalents in nor-

Fig 146 Diakinesis of barley showing translocation cross involving chromosomes 4 and 5 (Courtesy of Mrs. Christine E. Fastnaught McGriff Department of Plant and Soil Science, Montana State University Bozeman, MT)

mal meiosis have only two centromeres that are arranged in coorientation and that distribute the two chromosomes involved to opposite poles However, several types of orientation in the metaphase plate are possible when a quadruple is formed since not two but four centromeres are involved

Theoretically the following types of chromosome orientation are distinguished

1 Coorientation
   a alternate-1 orientation
   b alternate-2 orientation
   c adjacent 1 orientation
   d adjacent 2 orientation
2 Noncoorientation

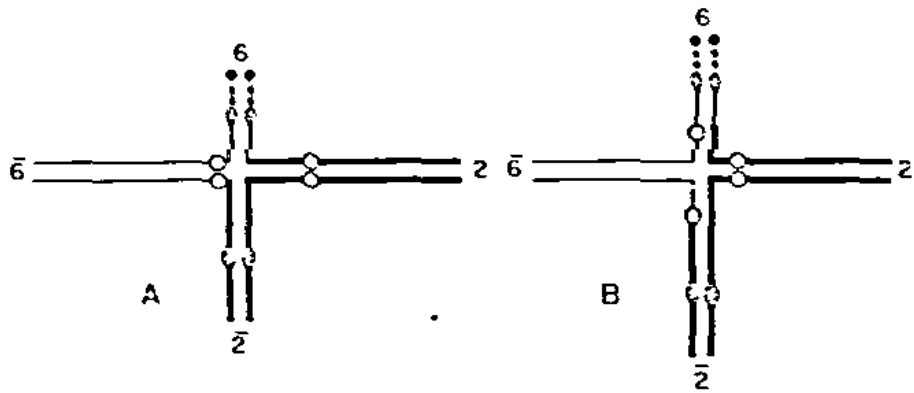


Fig 147A and B Line interpretations show synapsis in two different positions of the center of the cross of the T2 6a translocation heterozygote in maize The thin lines represent chromosomes 6 The thick lines chromosomes 2. (*A*) Association of homologous parts in the center of the cross at the original exchange breakpoints. (*B*) Association of nonhomologous segments in the center of the cross not at exchange points. (From Burnham 1962 Redrawn by permission of Charles R Burnham St. Paul Minnesota)

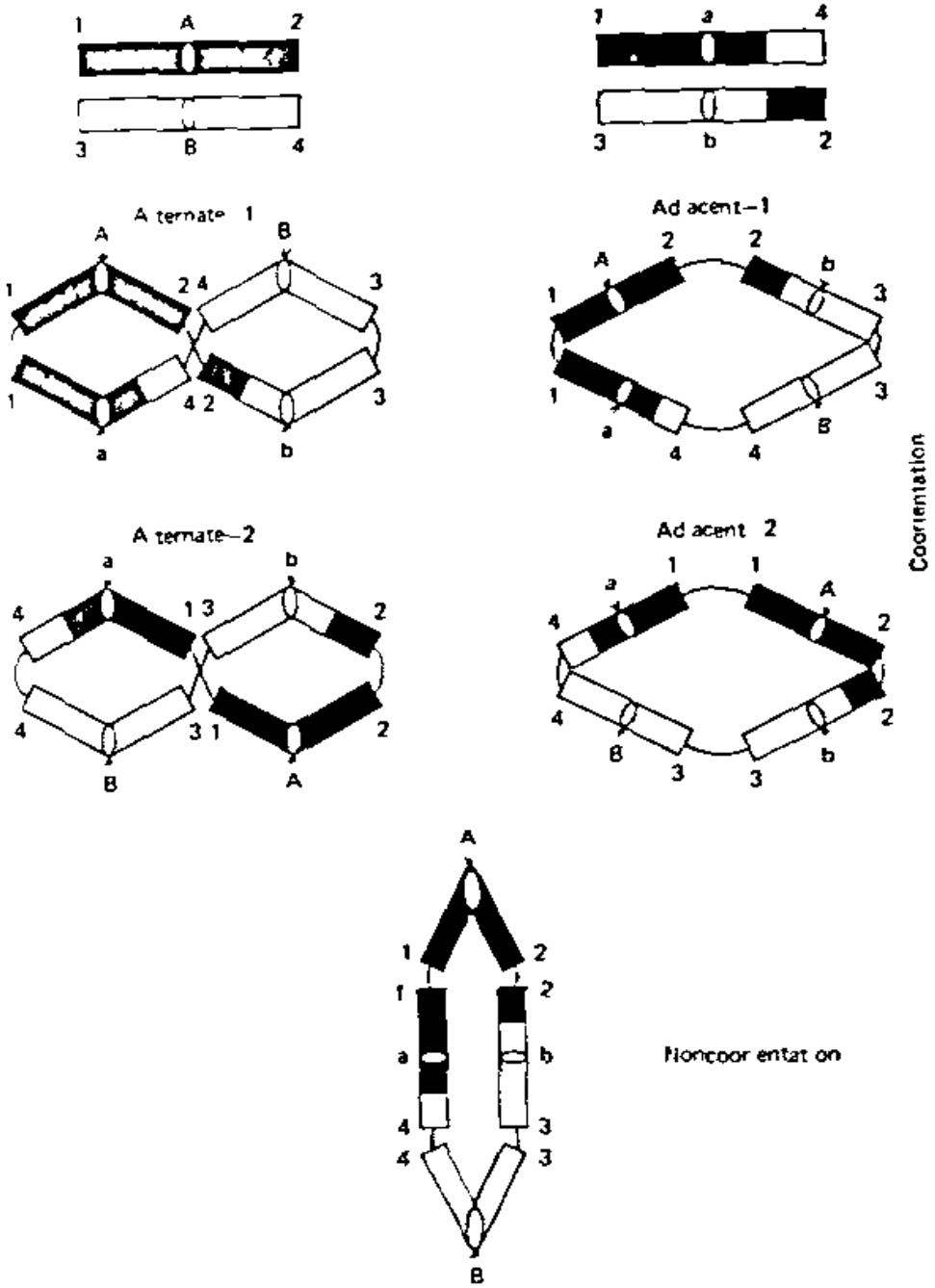


Fig 14.8 Orientation types of quadruples in metaphase I Numbers designate chromosome ends and letters designate centromeres Explanation in text

Chromosomes of quadruples in coorientation generally are distributed in even numbers to opposite poles In alternate 1 and adjacent 1 orientation, homologous centromeres (A,a, Fig 14 8) coorient and wander to opposite poles just like during normal bivalent separation In alternate-2 and adjacent 2 orientation, nonhomologous centromeres (A b) coorient and pass to opposite poles (Fig 14 8) Appar ently there is no guarantee in quadruples as there is in bivalents, that homologous centromeres coorient and go to opposite poles Consequently, alternate 2 and adjacent 2 orientations actually occur Endrizzi (1974) could morphologically dif ferentiate all four coorientation types in three different translocations of cotton

In all three the ratio of alternate-1 to adjacent-1 was 1 1 In one translocation the ratio of alternate 2 to adjacent 2 was 1 1 but in another 2 1

In order that balanced combinations result in quadruples, alternate orientation has to occur Only if the two translocated chromosomes (centromeres a and b, Fig 14 8) pass to one pole and the two nontranslocated chromosomes pass to the other (centromeres A and B) will there be balanced chromosome complements in the gametes This condition is met *in the alternate-1 and alternate 2 orientations* indicated in Fig 14 8 All gametes that do not meet this condition will have duplicated and deleted chromosome complements that are caused by adjacent 1 and adjacent 2 orientation In the case of adjacent-1 in Fig 14 8, one gamete will be duplicated for segment 2 and deficient for segment 4 and the other gamete will be duplicated for segment 4 and deficient for segment 2 In the case of adjacent-2 one gamete will be duplicated for segment 1 and deficient for segment 3, while the other gamete will be duplicated for segment 3 and deficient for segment 1 Such gametes are also referred to as **Dp-Df gametes** (duplication-deficiency) In general, **semisterility** results in translocation heterozygotes because of the formation of balanced and unbalanced gametes The proportion of fertile and aborted gametes is close to 1 1 in several species This is expressed, for instance, in half of the seeds missing in an inflorescence of a plant Examples of such plants are maize, petunia, peas, and sorghum In mammals, reciprocal translocations have been investigated in mice Varying degrees of semisterility were demonstrated by Carter et al (1955), Ford et al (1956) and Shizinsky (1957) In humans the frequency of chromosome imbalance among reciprocal translocation progeny is likely to be less than the theoretical 50% (Hamerton, 1971a)

However, it has been observed that there is **preferential or directed segregation** of quadruple chromosomes in some species In these instances alternate orientation and disjunction of chromosomes range from 70% to 95% Examples of directed segregation are *Hordeum Secale, Datura, Triticum, Oenothera* and several insects

Species that have directed chromosome segregation seem to meet some specific *requirements for movability as far as the chromosomes are concerned* Factors that seem to influence quadruple orientation are

1 length of the chromosomes
2 *position of the breakpoints*
3 number and position of chiasmata
4 degree of chiasma terminalization
5 position of centromere

Alternate segregation seems to be increased if the chromosomes involved in the quadruple are uniform in length Also, short chromosomes are easier to maneuver on the metaphase plate If chromosomes are too short, they are often too rigid for alternate orientation In *Oenothera* for instance, the chromosomes are all of the same length

The position of the breakpoints seems to influence chiasma formation in the quadruple It is generally believed that chiasma formation is reduced in the interstitial segments (between centromere and breakpoint) (Sybenga, 1972) This partially may be the case because heterochromatin *near the centromeres does not allow* active pairing activity, which is a prerequisite for crossing over and chiasma for-

Fig 14 9 Noncoorıented quadruple ın a T5-7g translocatıon heterozygote of barley (× 2244) (Schulz Schaeffer, unpublished)

mation If the breakpoints are distal, the ınterstıtıal segments are large, and crossıng over ıs reduced to a mınımum Thıs reduces the number of chıasmata, and terminalızation can be accomplished by metaphase I If no ınterstıtıal chıasmata are present by metaphase I, the quadruple will be quite maneuverable on the metaphase plate *It ıs obvious that a medıan posıtıon of the centromere also would* enhance flexibility of the quadruple

In noncoorientation (Fig 14 8) the two centromeres on opposite sides of the quadruple (e g , A, B) are cooriented and are positioned equidistant from the equatorial plate The two intermediate centromeres (e g , a,b) are noncooriented and are stretched out between the other two, seemingly not attached to the poles by centromeres Figure 14 9 shows a noncooriented quadruple ın barley In anaphase I the two cooriented chromosomes pass to opposite poles while the non cooriented ones either pass to the same pole (3 1 segregation) or pass to opposite poles (2 2 segregation) In 3 1 segregation of the quadruple the gametes become aneuploid (Fig 14 10) After fertilization, this leads to trisomy or monosomy (Chapter 16) Noncoorientation always will lead to unbalanced gametes In the case of 2 2 segregation, normal and translocated chromosomes will pass to the same poles (e g , A, a) and duplication-deficiency gametes will result, as ın adjacent orientation

Another centromere orientation phenomenon ıs **reorientation.** Often the ınıtıal orientation at the metaphase plate ıs not appropriate, and, therefore, reorientation ıs necessary for controlled chromosome segregation Thıs may mean the loss of a chromosomal spindle fiber connection to one pole followed by the formation of a new connection to the opposite pole (Rieger et al , 1976) Such a phenomenon has been observed ın *Tipula oleracea* ın lıvıng and fixed materıal (Bauer et al , 1961, *Rohloff, 1970)*

14.4 Translocations in Humans

A prominent structural change ın human populations ıs the recıprocal transloca tion (Hamerton, 1971b) Sınce meıosıs ıs not readily accessible for study ın humans, *the spotting of recıprocal translocations* as translocation quadruples *is*

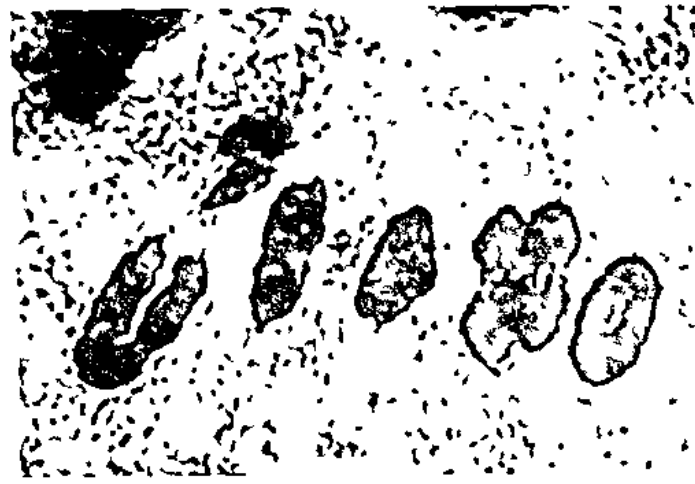


Fig. 14.10. Segregation of a quadruple in a T5-7g translocation heterozygote of barley (× 2231) (Schulz-Schaeffer unpublished)

not a good scoring method. Consequently translocations in humans have been mainly detected by karyotyping. The modern methods of chromosome banding have been extremely helpful in this respect (Section 2.3). A frequent structural rearrangement in man is the so-called **Robertsonian translocation** or centric fusion type (Robertson, 1916). This was confirmed through a cytogenetic survey of 11,680 *newborn infants* by Jacobs et al. (1974). These findings verified the earlier observation by Court Brown et al. (1966) that Robertsonian translocations ranked highest in a high risk population including state hospital patients and *patients attending a subfertility clinic*. They had studied *1,870 individuals*, and the frequency of Robertsonian translocations was 0.43%, while that of other reciprocal translocations was 0.16%. Other reports of Robertsonian translocation were by Hamerton et al. (1961), Kjessler (1964), Hamerton (1966), and Hultén and Lindsten (1970).

A Robertsonian translocation is the centric fusion between two acrocentric chromosomes, which results in the reduction of the chromosome number (2n = 45). The least complicated way would be an interchange between the long arm acentric fragment of one chromosome and the short arm acentric fragment of the other chromosome (Fig. 14.11). The two acrocentric chromosomes break close to the centromere. The two long arms fuse and result in a metacentric chromosome. The two short arms form a very small chromosome that may be lost without any genetic damage to the organism. The reason that such breakage occurs more frequently here than at other parts of the chromosome lies in the inherent nature of heterochromatin. Since heterochromatin is located close to the centromeres, breakage happens more often in that region (Section 2.2.1). This phenomenon is remindful of the findings of McClintock (1950a, 1950b, 1951, 1953) who demonstrated a close association of heterochromatin with chromosome breakage (Section 12.3.1).

The human chromosomes most often involved in Robertsonian translocations are the acrocentrics of the D group (13 to 15) and of the G group (21 and 22). They

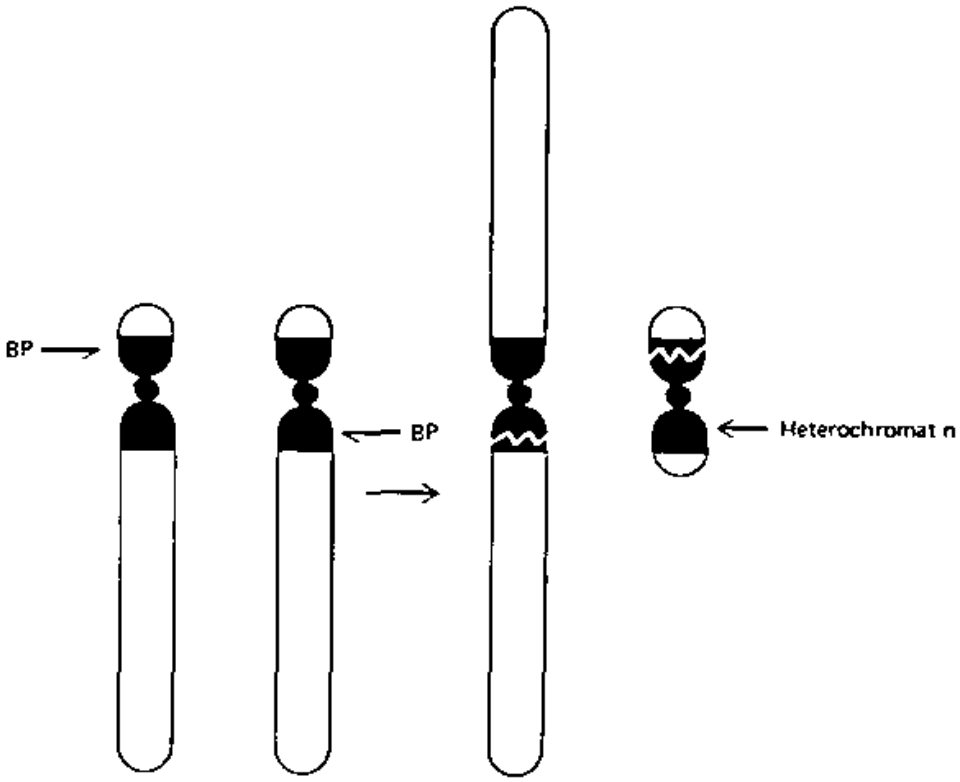


Fig 14 11 Possible explanation of a Robertsonian translocation Breakpoints (BP) occur in the heterochromatic regions close to the centromere in the short arm and long arm respectively of two acrocentric chromosomes Translocation products are a long armed metacentric chromosome and a very small metacentric chromosome that may be lost without genetic damage to the organism

are usually closely associated with each other in the cell because they are the organizers of the nucleoli that fuse during the prophase of meiosis (Section 7 2) The close proximity of these chromosomes favors interaction when lesions occur Since the D and G chromosomes are involved in Robertsonian translocations of humans Patau (1961) designated them as D/D, D/G or G/G translocations, and this nomenclature has since been restricted to such types of **centric fusion** Apparently the most frequent reports of centric fusion type translocations are the D/G type The reason for this is the association of this translocation with an increase in frequency of children with Down's syndrome who can be easily identified morphologically (Court Brown, 1967) The most common D/G centric fusion seems to be the one between chromosomes 14 and 21 (Hamerton 1971b) If a Robertsonian translocation occurs between chromosomes 14 (D group) and 21 (G group) in humans, the result will be three chromosomes that can associate in meiosis, the translocated chromosome 14^{21} and the two nontranslocated chromosomes 14 and 21 If regular coorientation occurs, a trivalent will separate in such a fashion that the central 14^{21} chromosome will pass to one pole while the other two chromosomes pass to the opposite one (Fig 14 12) Such disjunction will result in balanced gametes since each will receive the essential parts of both chromosomes 14 and 21 However, if nondisjunction will occur, two adjacent

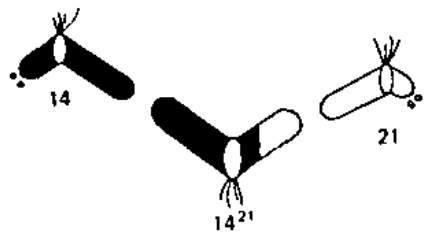


*Fig 14 12 Metaphase I configuration of a tri*valent formed by human translocated chromosome 14^{21} and two nontranslocated chromosomes 14 and 21

chromosomes ($14+14^{21}$ or $14^{21}+21$) can pass to the same pole If a gamete with such a combination gets fertilized it will result in a zygote that carries the equivalent of an extra chromosome, though the chromosome number will be normal ($2n=46$) Such cases have often been recorded as trisomics but they are not termed right Down s syndrome results from the $14^{21}+21$ combination Since the duplicated element is the long arm of the smaller chromosome 21, the chromosome abnormality can survive more readily Another type of Robertsonian translocation has been reported between the short arm of the Y chromosome and the long arm of chromosome 15 (Šubrt and Belhova, 1974) in four generations of male progeny Balanced chromosome polymorphism for Robertsonian translocations also has been reported in animals such as the goat (Soller et al , 1966), European wild pig (McFee et al , 1966), house mouse (Evans et al , 1967, Léonard and Deknudt, 1967, White and Tjio, 1968), and cattle (Gustavsson, 1966, Gustavsson et al , 1968)

Heterozygotes must produce balanced gametes in most of these instances of Robertsonian translocation heterozygosity in humans and animals since semisterility is rarely reported A balanced translocation of part of the long arm of chromosome 13 [13q (q21-qter)] attached to the long arm of chromosome 4 (4 q-) is shown in Figs 14 13 and 14 14 (Vigfusson, unpublished) This mother was normal because of the balanced condition But her child had a displaced duplication (Section 12 1) resulting in two normal 13 chromosomes and a duplicated 13q21-13qter segment attached to chromosome 4, inherited from the mother

Other types of reciprocal translocations in humans have been recorded such as t(A,A) between two of the 3 group A chromosomes by Lee et al (1964), Summitt (1966) and Lejeune et al (1968b) others between chromosomes of the A group and those of the B group, or t(A,B), by Court Brown et al (1964), De Grouchy (1965), De Grouchy et al (1966) and by Walzer et al (1966) Many other combinations (A C, A,G, B,B, etc) are possible Many of these cases were found because the patient was mentally retarded or had congenital malformation

Two recent discoveries of 46 chromosome reciprocal translocations are a t(B,D) involving a translocation of the distal half of the long arm of chromosome 14 onto the short arm of chromosome 5 (Fig 14 15) and a t(A,C) involving a translocation of most of the long arm of chromosome 2 onto the long arm of chromosome 8 (Fig 14 16) Both photographs indicate the usefulness of modern banding techniques in identifying translocations The fibroblast cell cultures in these two cases were established from skin biopsies of a 17 year-old normal male who presented emotional and mental problems and from a 24-year-old normal male who was a bal-

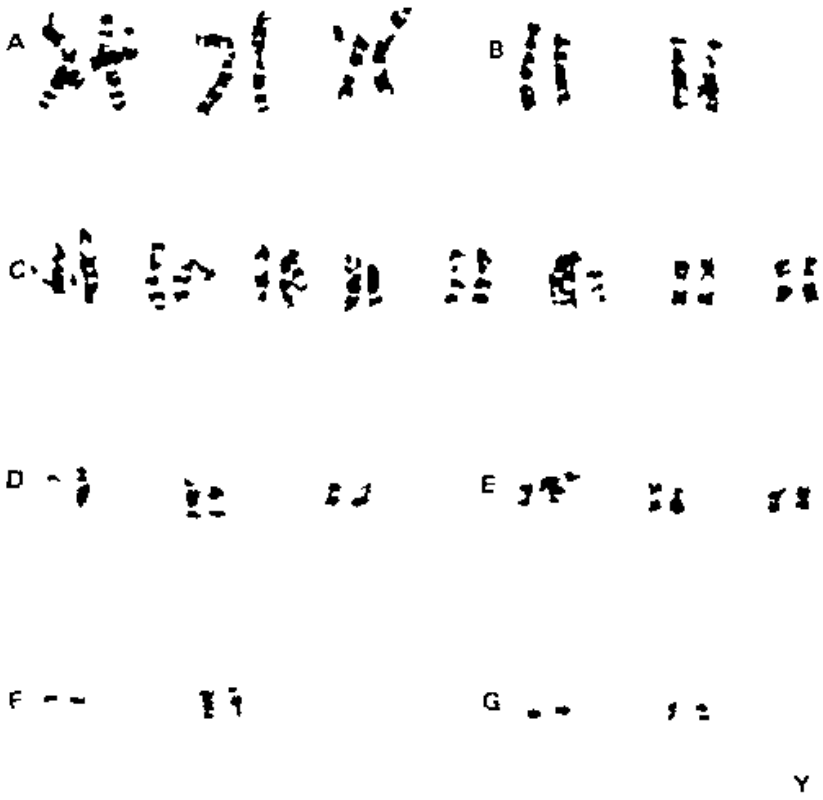


Fig 14 13 Karyotype of a human with a balanced translocation of part of the long arm of chromosome 13 attached to the long arm of chromosome 4 (Courtesy of Dr Norman V Vigfusson, Department of Biology, Eastern Washington University, Cheney)

anced translocation carrier, respectively Stocks of both are stored in the **Human Genetic Mutant Cell Repository** at the Institute for Medical Research, Camden, New Jersey

Every arm of the human chromosome complement has been reported as being involved in chromosome translocations (Absate and Borgaonkar, 1977) By 1977 at least 490 so-called "simple translocations," 224 reciprocal translocations, 84

Fig 14 14 Photomicrographs of human chromosome pairs 4 and 13 showing reciprocal translocation involving one chromosome of each pair (Courtesy of Dr Norman V Vigfusson, Department of Biology, Eastern Washington University, Cheney)

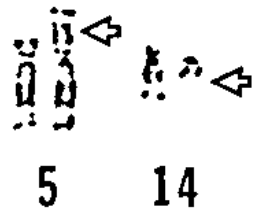


Fig 14 15 Photomicrographs of human chromosome pairs 5 and 14 showing a reciprocal translocation of the distal half of the long arm of chromosome 14 onto the short arm of chromosome 5 (From Borgaonkar et al 1977 Reprinted by permission of S Karger AG Basel)

Robertsonian translocations 24 tandem translocations and 15 complex translocations have been reported in human chromosomes (Borgaonkar 1977)

14 5 Complex Heterozygosity

The possibility of a reciprocal translocation changing the meiotic behavior of an organism was discussed in Section 14 3 This will be even more drastic if more than one translocation has occurred Figure 14 17 shows two rings of 4 chromosomes in barley where two reciprocal translocations are involved The mechanics of events leading to **complex heterozygosity** are best explained in the case of two reciprocal translocations leading to the formation of a **hexaple**, the equivalent of a quadruple, but where six chromosomes are united in a pairing configuration instead of only four (Fig 14 18) Two reciprocal translocations in the same chromosome complement form such a configuration of six chromosomes if they involve the same chromosome pair *This happens if a reciprocal translocation occurs between a member* of a ring of four chromosomes and another chromosome pair In this case it is not important if the two translocations involve the same chromosome (Fig 14 18A) or both homologous chromosomes of the same pair (Fig 14 18B) In Fig 14 18A, chromosomes A(1 2), B(3 4) and C(5 6) are the nontranslocated chromosomes The capital letters designate the chromosomes, while the numbers designate the chromosome arms Chromosomes A′(3 6), B′(1 4) and C′(5 2) are the translocated chromosomes where A′ is involved in two translocations With alternate-1 disjunc

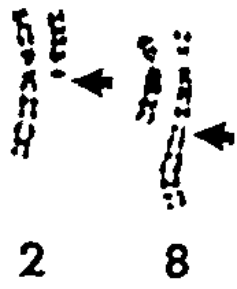


Fig 14 16 Photomicrographs of human chromosome pairs 2 and 8 showing a reciprocal translocation of most of the long arm of chromosome 2 onto the long arm of chromosome 8 (From Worton et al , 1977 Reprinted by permission of S Karger AG, Basel)

Fig 14 17 A metaphase I cell of a translocation heterozygote of barley The two rings of 4 chromosomes each indicate two reciprocal translocations (3^{II} and 2^{IV} n=7) (Courtesy of Mrs Christine E Fastnaught McGriff, Department of Plant and Soil Science, Montana State University, Bozeman)

tion (homologous centromeres to opposite poles A,A′, etc), the translocated chromosomes (A′, B′, C′) all pass the the same pole, and complete compensation for displaced chromosome ends guarantees fertile gametes In Fig 14 18B, the two translocations are shared by both partners of a homologous pair (A, A′) There are four translocated chromosomes, A(1 6), A′(2 3), B′(1 4), C′(2 5), and two nontranslocated ones, B(3 4) and C(5 6) Even though translocated chromosomes pass to both poles in alternate-1 disjunction, complete compensation for displaced chromosome ends is guaranteed also in this instance Tuleen (1972) found rings of six chromosomes in barley intercrosses and could demonstrate by locating the breakpoints that the two translocations involved the same chromosome

If a third translocation involves a chromosome of a hexaple, a ring of eight chromosomes can occur This process can continue until all chromosomes of the complement are involved in what is known as a translocation complex

The best known case of **complex heterozygosity** is the genus *Oenothera* (Cleland, 1962, 1972) Here, not only forms with rings of 6 chromosomes are present but also with rings or chains of 8, 10, 12, and even all 14 chromosomes

14 6 *Oenothera* Cytogenetics

Oenothera (2n=14) is one of several plant genera that has developed mechanisms favoring the formation and frequency of translocation heterozygotes in the population Renner (1914, 1917) first discovered that in this genus "there are several different **genetic factor complexes** which are combined in pairs in the various species and these complexes segregate as wholes in meiosis, each gamete carrying one or the other " They were named after him—**Renner complexes.** Prior to that in 1908, Gates had first observed **multiples,** chromosome associations of more than two, in *O rubrinervis* Belling (1925, 1927) in his **interchange hypothesis** concluded that the multiples had to be the result of chromosome translocations This explained the cytological nature of the Renner complexes Within the pairing configuration of *O lamarckiana,* for instance, the paternal and maternal chromosomes are arranged alternately joined together by reciprocal translocations In

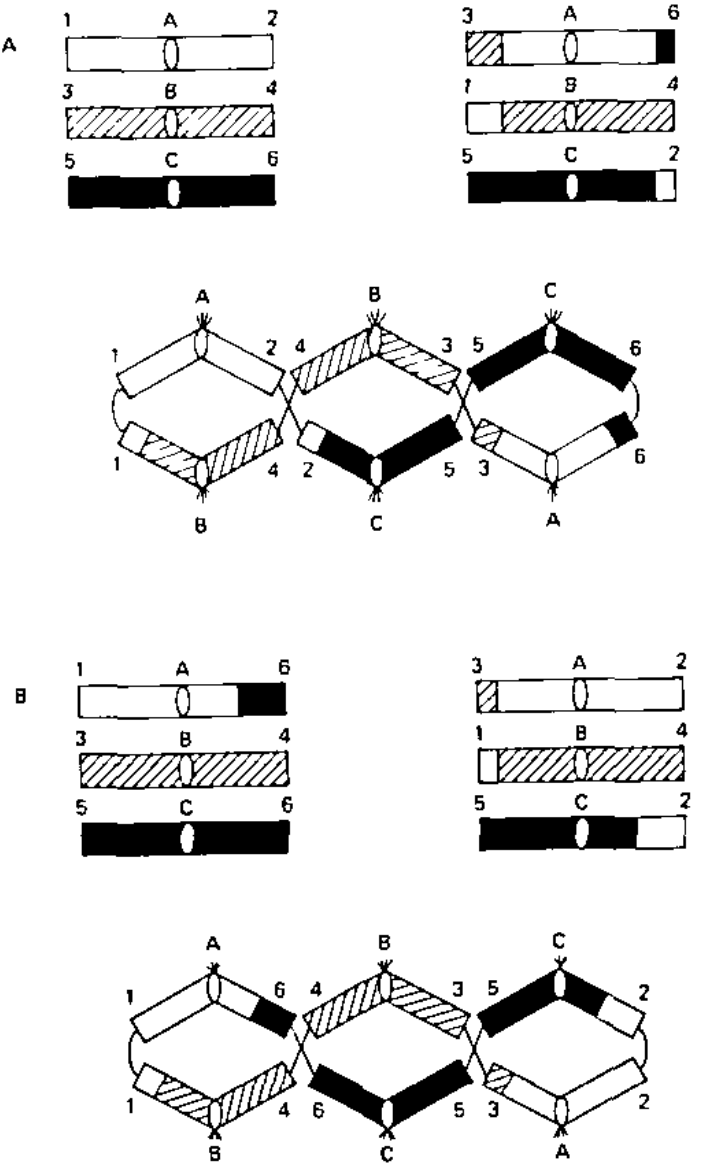


Fig 14 18A and B Diagrammatic representation of 2 reciprocal translocations involving 3 chromosome pairs leading to a ring of 6 chromosomes in metaphase I (*A*) Two translocations involve the same chromosome (3 6) (*B*) Two translocations involve both chromosomes of a homologous pair (1 6 3 2)

metaphase I, they are arranged in alternate orientation so that eventually one pole receives all the paternal chromosomes while the other one receives only the maternal ones Consequently, only two kinds of gametes result that are identical to those from which the plant was formed The complex is comprised of those chromosomes that are distributed in meiosis as a unit (Fig 14 19) In *O lamarckiana* the two complexes that segregate to opposite poles in meiosis are called *gaudens* and *velans* On outcrossing to other forms, it became evident that the *gaudens* complex carries genes for red spots on leaves that are broad and express nonpunctate stems

Fig 14 19 Complex heterozygosity in *Oenothera* Two Renner complexes (white from father black from mother) are distributed in anaphase I to opposite poles (From Cleland, 1962)

and green buds The *velans* complex possesses genes that prohibit the expression of red spots on leaves that are narrow and express punctate stems and red striped buds But self fertility in these species guarantees maximal heterozygosity and that the complexes do not break up but stay together **Balanced lethals** (Muller 1917) insure that only heterozygotes are formed Such a system consists of two or more linked recessive lethal genes that are permanently maintained in the heterozygous condition such as l L /L l All homozygotes abort because of the double recessive lethal effect (e g l L /l L_2 or L l_2/L l_2) In *O lamarckiana* for instance both megasporocytes and microsporocytes develop *gaudens* and *velans* gametes (Fig 14 20) During the formation of zygotes only the *gaudens velans* (G V) zygotes survive, while the *velans velans* (V V) and *gaudens gaudens* (G G) zygotes abort This phenomenon is known as **zygotic lethality** In *O muricata* the two complexes are called *rigens* (R) and *curvans* (C) Here the inactivation occurs earlier during gametogenesis already In male gametogenesis the game

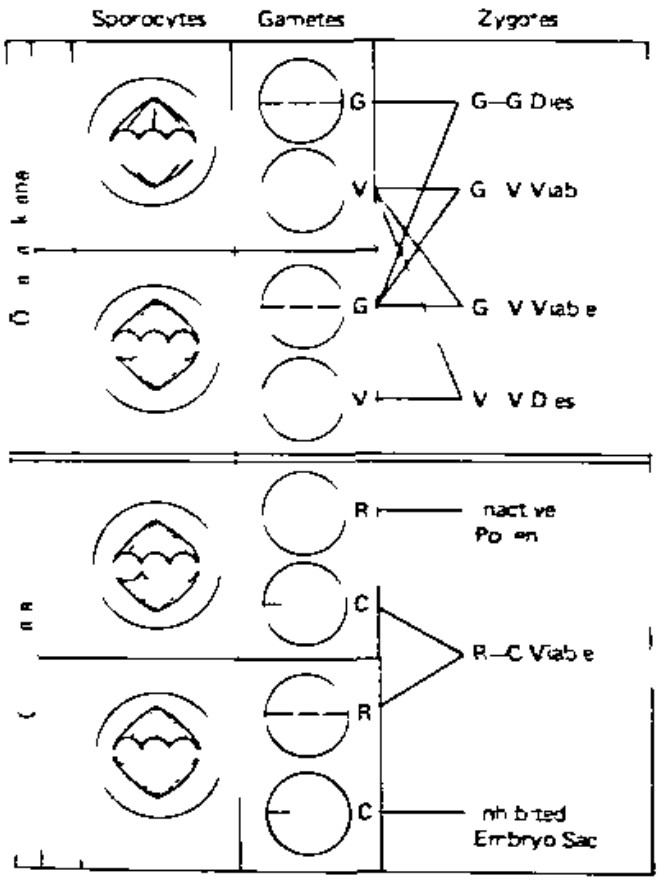


Fig 14 20 Diagrammatic representation of zygotic lethality in *Oenothera lamarckiana* and of the Renner effect in *O muricata*. Explanation in text. (From Swanson, 1957 Redrawn by permission of Prentice Hall, Inc., Englewood Cliffs NJ)

tophytes carrying the *regens* complex do not survive so that only *curvans* (C) pollen are effective In female gametogenesis the gametophytes carrying the *curvans* complex do not survive and only *rigens* embryo sacs develop Consequently only *rigens-curvans* (R-C) zygotes form This phenomenon, a case of megaspore competition was also discovered by Renner (1921) and was called the Renner effect by Darlington (1932) In summary, then the devices that guarantee complex heterozygosity in *Oenothera* are

1 reciprocal translocations
2 balanced lethals
3 self pollination

14.7 Other Systems with Complex Heterozygosity

Another well investigated system of complex heterozygosity is the genus *Rhoeo* (2n = 12) Studies in this genus have been performed by Darlington (1929a, 1929b), Kato (1930), Sax (1931), Anderson and Sax (1936) Simmonds (1945), Tschermak-Woess (1947), Walters and Gerstel (1948), Stearn (1957) Flagg (1958), Carniel (1960), and Wimber (1968) In contrast to *Oenothera* the chromosomes in this system are not all of equal length Sax presented an idiogram that was prepared from mitotic chromosomes (Fig 14 21) The idiogram also demonstrates that the positions of the centromeres in *Rhoeo discolor* are not median in every instance This may have resulted from unequal reciprocal translocations Sax concluded that there were translocated chromosomes in both complexes He designated the arms of the nontranslocated chromosomes with the same letter, but the left arm with a capital letter and the right arm with a small letter (e g , Aa) DE is an example of a translocated chromosome that possesses the left arm (or any portion thereof) of an originally nontranslocated chromosome Dd and the right arm (or any portion thereof) of an Ee chromosome In the metaphase I ring formation, the 12 chromosomes will be attached to each other in the following way Aa aB-Bb-bC-Cc-cD-DE-Ee-ed-dF-Ff-fA (where fA is also attached to Aa) If these chromosomes are arranged in alternate or zig zag formation then the two complexes pass to opposite poles, each gametic complex con-

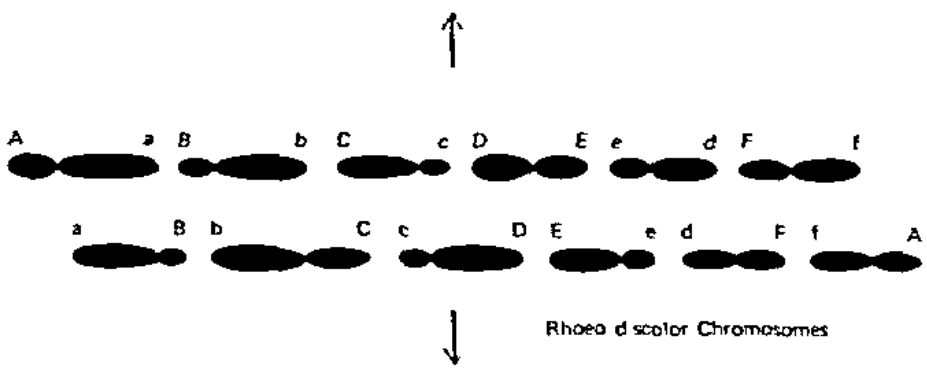


Fig 14 21 The two Renner complexes of *Rhoeo discolor* (Adapted after Sax, 1931 Redrawn by permission of Cytologia, Tokyo)

Complex heterozygosity also can be produced experimentally For instance Yamashita (1947 1950, 1951) produced various translocations in diploid *Triticum* species by x ray treatment After crossing different homozygous translocation lines he was successful in establishing a line in which all 14 chromosomes were united in one translocation complex Similar results were accomplished in *Campanula Hordeum Tradescantia* and *Zea* (see review by Burnham 1956) Darlington and LaCour (1950) produced a system of translocation heterozygosity in *Campanula persicifolia* in which all chromosomes of this species were involved *In most of these* systems total sterility results because alternate orientation usually does not occur regularly

14 8 Chromosome Mapping via Translocations

The translocation itself behaves like any other genetic factor Its genetic expression semisterility, shows linkage with genes in two different linkage groups and behaves like a dominant character in test crosses In 1930 Burnham found linkage between the gene *wax* (*wx* 59, chromosome 9) and partial sterility caused by translocation in maize

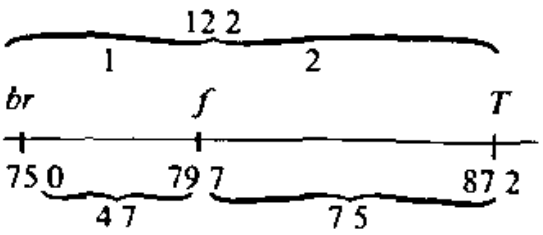
The genetic expression of semisterility will be demonstrated here with the data of Brink and Cooper (1931) obtained from a linkage test involving semisterile-1, *brachytic* (*br* 75) and *fine striped* (*f* 80), both located on chromosome 1 of maize Semisterile 1 plants $\left(\frac{+\ +\ T}{br\ f\ +}\right)$ were backcrossed to nontranslocated plants $\left(\frac{br\ f\ +}{br\ f\ +}\right)$ as shown in Table 14 1 (see p 240) in typical testcross fashion A 1 1 ratio of semisteriles (SS) to fertiles (F) would have been expected in each of the four segregation classes if semisterility had been independent from *br* and *f* Consequently, the three-point cross data show linkage with semisterility Semisterility has all the characteristics of a gene located at the translocation breakpoint Its location in relation to the other two genes can therefore be determined and mapped (Table 14 1) The location of *br* and *f* is known from other studies Since *br* is located at position 75 0 and *f* at 79 7, the translocation point must be located distal from these at 87 2 since its position is 12 2 units from *br* and 7 5 units from *f* The method of determining linkage between translocation breakpoints and new genes has been used profitably for assigning these genes to specific chromosomes or even chromosome arms or segments

In Chapter 4 (Section 4 4) it was indicated that translocations can serve for the determination of gene position Burnham (1957, 1962, 1966) described two methods, the **all-arms marker method** (Burnham and Cartledge, 1939, Burnham, 1954) and the **linked marker method** (Anderson 1943, 1956) *In the all arms* marker method, a tester set was developed in maize that included at least two translocation breakpoints in every arm of the complement (Burnham 1966) The "all-arms tester set" *included 22 translocations by 1962 (Burnham) Plants of a* strain with an unmapped gene or a new mutant are being crossed with the trans-

Table 14 1 Three point cross Linkage test involving semisterility in chromosome 1 of maize (Brink and Cooper, 1931)

	$\frac{+\ +}{br\ f}$	$\frac{+\ f}{br\ f}$	$\frac{br\ +}{br\ f}$	$\frac{br\ f}{br\ f}$	Totals
SS[1]	333	1	17	25	376
F[2]	19	8	6	273	306
					682
					Percent
Noncrossovers					
$+\ +\ T$	333				
$br\ f\ +$	273		606		88 8
Single crossovers in region 1					
$br\ +\ T$	17				
$+\ f\ +$	8		25		3 7
Single crossovers in region 2					
$+\ +\ +$	19				
$br\ f\ T$	25		44		6 5
Double-crossovers					
$+\ f\ T$	1				
$br\ +\ +$	6		7		1 0
Total			682		100 0

[1]SS = semisterile plants
[2]F = fertile plants



1 2 = crossover regions 1 and 2

location lines of the tester set The semisterile F_1 is backcrossed to the parental stock with the recessive mutant character The progeny is classified for semisterility of the pollen and for the mutant If there is no linkage the percentage of mutants should be similar in the fertile and semisterile classes A higher percentage of mutants in the fertile class is an indication of linkage between the mutant gene and the translocation point

In barley, too, translocations served to determine linkage groups to which new genes belong (Swomley, 1957, Ramage and Suneson, 1958, Ramage, 1964) But first of all, in barley, translocations helped to assign linkage groups permanently to chromosomes For instance, two linkage groups formerly thought to be independent (III and VII, Robertson et al , 1941) were both demonstrated to be on the newly assigned chromosome 7 (Kramer et al , 1954) They used linkage tests (F_2 data) from crosses between translocation stocks and genetic markers

A tester series of translocations linked with the endosperm marker is an efficient method of locating genes In one series in maize, translocations are used where one breakpoint is close to the *waxy* locus (*wx* 59 0) in the short arm of chromo-

Part VII
Variation in Chromosome Number

Chapter 15
Haploidy, Diploidy, and Polyploidy

15.1 Haploidy

Haploidy is a general term for designating individuals or tissues (in mosaics) that have somatic cells with a gametic chromosome number (n) However, since particularly in plants, polyploid series occur that carry multiples of the basic chromosome set (x) the term *haploidy* should be subdivided into two major categories

1 **monohaploids** (x) individuals that can arise from diploid species
2 **polyhaploids** (2x 3x 4x, etc) individuals that can arise from any given polyploid species (4x → 2x 6x → 3x etc)

If this relationship is understood, the term haploid is still a very useful, general term that can be used in discussions of this topic

15 1 1 Origin of Haploids

Haploids can arise spontaneously or can be induced The origin of spontaneous haploids is often obscure They have occurred from time to time and have been reported in the literature They usually arise by asexual development as a haploid of an individual that should be diploid (Chapter 19)

Among the animals, haploids have frequently been discovered in *Drosophila* (Castle, 1934, Bridges, 1925) Other references on spontaneously and induced occurring haploidy in animals are salamander (Fankhauser, 1937), newt (Fankhauser and Griffiths, 1939), frog (Briggs, 1952), mouse (Edwards, 1954), axolotl (El'Darov, 1965), Anura (Hamilton, 1966), chicken (Bloom, 1970), and onionfly (Heemert, 1973) Usually in animals, haploidy produces physiologically abnormal individuals that die during embryogenesis

Spontaneous plant haploids have been found in tomatoes (Morrison, 1932), and cotton (Harland, 1936) and more recently in coffee (Visheveshwara, 1960), beets (Fisher 1962), barley (Tsuchiya, 1962), flax (Plessers, 1963), coconut (Ninan and Raveendrananatth, 1965), pearl millet (Powell, 1969), rape (Thompson, 1969, Stringham and Downey, 1973), *Theobroma* (Dublin, 1973), asparagus (Marks, 1973), and wheat (Lacadena and Ramos, 1968, Sendino and Lacadena, 1974)

Kimber and Riley (1963) reported 36 species of 26 genera and 10 families in which haploidy occurred spontaneously

Several methods of obtaining spontaneous and induced haploids have been described in the literature They are

1 interspecific and intergeneric hybridization
2 irradiation and chemical treatment
3 selection of twins
4 alien cytoplasm
5 isolation following pollination involving a pollen or seed parent carrying a marker
6 anther and pollen culture
7 chromosome elimination

One of the first accounts of experimental results on haploid production following hybridization was given by Jørgensen in 1928 He crossed *Solanum nigrum* with *S luteum* and 7 of the 35 resulting plants were *S nigrum* haploids This was an example of **matroclinal pseudogamy** for which a male gamete was required for endosperm formation to stimulate embryo development However, the embryo was developed directly from the egg without fertilization Chase (1947, 1949a, 1949b, 1949c, 1952a, 1952b) and Coe (1959) isolated male stocks in wheat and maize which after intraspecific hybridization produced high frequencies (2% to 3%) of haploids in these species The method of interspecific hybridization found wide application in potato breeding The potato is thought by some to be an autotetraploid ($4x=48$) Diploidy in this species would offer less complicated inheritance ($2x=24$) Hougas et al (1958, 1964) reported a method by which they could produce high frequencies (tenfold increase) of haploids and also could spot them easily by using a pigmented pollinator They crossed tetraploid *Solanum tuberosum* with diploid *S phureja* All the nonpigmented plants were suspected haploids Other reports on the success of this method were by Frandsen (1967), Budrin (1969), and Cipar and Lawrence (1972) Reviews on the subject of interspecific and intergeneric crossing for haploid production were carried out by Magoon and Khanna (1963), Kimber and Riley (1963), and Chase (1969) A total of 39 species were reported by Rowe (1974) to produce haploids after wide hybridization

The method of x irradiation for haploid production has been tried by various researchers in several crops A few should be mentioned such as the work on tobacco (Goodspeed and Avery, 1929, Webber, 1933, Ivanov, 1938, Badenhuizen, 1941), wheat (Katayama, 1934, Yefeiken and Vasilev, 1936), *Crepis* (Gerassimova, 1936a, 1936b), snapdragon (Ehrensberger, 1948), and *Oenothera* (Linnert, 1962) In the experiments of Yefeiken, Ehrensberger, and Linnert, normal plants were pollinated with irradiated pollen Some irradiated pollen must lose its ability to fertilize, thus stimulating the unfertilized egg to parthenogenetic development Gamma radiation treatment of pollen for the production of haploidy in poplar trees has also been reported (Winton and Einspahr, 1968, Stettler, 1968)

Induction of haploidy by treatment of pollen with a vital dye, toluidine blue (TB) has been reported for *Vinca rosea* (Rogers and Ellis, 1966), tomato, maize (Al-Yasari, 1967, Al-Yasari and Rogers, 1971), and poplar (Winton and Stettler, 1974) But even greater success was reported when the TB treatment was applied to the pistils after pollination, at a time when the pollen tubes had developed but

had not engaged in fertilization With such postpollination spray treatment 282 maternal haploid seedlings were scored from a total of 1 192 seedlings raised (23 6%) (Illies 1974)

The method of screening twin seedlings for the selection of haploids has been successful in quite a number of species Morgan and co-workers (Morgan and Rappleye 1950 1954 Campos and Morgan 1960) showed that the frequency of twin seedlings in *Capsicum* is controlled by the genotype of the female parent Through selection for favorable genotypes they were successful in raising the percentage of twins to a level of several percent in this species The frequency of haploids depends on that of the twin seedlings Such seedlings arise from **polyembryonic** seed Polyembryonic seeds can produce haploid haploid diploid-diploid or haploid-diploid twins It is believed that in haploid diploid twins a normal diploid zygote has developed together with a haploid synergid into two embryos Morgan and Rappleye found 30% haploid diploid twins among polyembryonic pepper seeds Results in other species are much lower Wilson and Ross (1961) found only 5% in bread wheat Lacadena (1974) reviewed the subject and reported the occurrence of twin seedlings for 42 plant species

The method of using alien cytoplasm as a means for the production of haploids was suggested by Kihara and Tsunewaki (1962) They backcrossed a hybrid *Aegilops caudata* x *Triticum aestivum* var *erythrospermum* with wheat and obtained a frequency of 53% haploids while no haploids were found in lines without cytoplasmic substitution The wheat with the *Aegilops* cytoplasm was obtained by this backcross method The method of haploid production that involves isolation of haploid material following pollination with a pollen or seed parent carrying a marker was already briefly mentioned in connection with the interspecific hybridization approach specifically in the potato program (Hougas et al 1958 1964) But this approach also has been applied in cases of intraspecific hybridization

Earlier studies with this method were carried out in maize Randolph and Fischer in 1939 used a seed parent with the genetic constitution A_1 *b pl* r^g y_1 and pollen with different genotypes to screen for parthenogenesis in tetraploid maize (2n=40) The anthocyanin *purple* plant color gene (*A* 111 chrom 3) requires the dominant *Booster* gene (*B* 49 chrom 2) and another dominant *anthocyanin* gene (*Pl* 48 chrom 6) to express purple plant color Since the seed parent carried both *b* and *pl* in the recessive condition the plants inherited green plant color The recessive genes for *colorless aleurone* (r^g 57 chrom 10) and for *white endosperm* (*y* 13 chrom 6) were also carried by the seed parent One of the pollen parents had the constitution *A B Pl* R^g Y_1 Any purple F_1 plants with colored aleurone and yellow endosperm were likely to be tetraploids Any green F_1 plants with colorless aleurone and white endosperm were parthenogenetic suspects or polyhaploids (2n=20) With a similar approach Randolph (1939) discovered 23 polyhaploid parthenogenotes among 17 165 tetraploid maize plants (1 750) Chase (1949a) found wide variation of haploid frequency in maize from 1 4 500 to 1 145 with an average of 1 900 The highest haploid frequency detected was 1 100 which is supposedly 20 times the average frequency in maize of 1 2000 (Stadler 1942) Seedling markers for haploid screening have been used for several other crop species In tobacco Burk (1962) used a recessive *yellow green* (*yg*)

seedling marker in the female parent In tomato, Ecochard et al (1969) used three recessive seedling markers In cotton, Turcotte and Feaster (1969) used several multiple gene markers either for the seed or pollen parent Bingham (1971) used hypocotyl pigmentation as a seedling marker in alfalfa to screen haploids in crosses between tetraploids and diploids

Anther and pollen culture methods for the production of haploids have recently been discussed by Sunderland (1974) and Nitsch (1974) Guha and Maheshwari (1964, 1966) are credited with the discovery of a method for the production of haploid plants directly from pollen by culturing anthers of *Datura innoxia* This work was followed up by the extensive work of J P Nitsch and his colleagues in France on tobacco (Bourgin and Nitsch 1967 Nitsch et al , 1968 Nitsch and Nitsch, 1969) Anthers in culture can yield haploid plants either by the direct formation of embryo-like products from pollen grains or by the formation of callus and subsequent plant regeneration Sunderland (1974) stated that simplicity of operation, ease of induction, and high induction frequencies are some of the merits of these methods He claims that given optimal culture conditions, induction frequencies of up to 100% can be obtained in *Datura* and *Nicotiana* In *Datura* haploid induction took place within 24 hours of culture (Sunderland et al , 1974) and in *Nicotiana* within several days (Sunderland and Wicks, 1971) The incidence of induction in a single anther in *Datura* was more than a thousand haploids and in *Nicotiana* even higher In *Nicotiana* and *Datura* the growth rate was comparatively high Production time from anther inoculations to mature plant stage was 3 to 4 months

Many other cultivated species have now been used for this kind of haploid production Some of these are mentioned in Table 15 1 It is important to notice that the bulk of the plants recovered from these experiments is not haploid The best results in this respect have been obtained with tobacco (Sunderland, 1970, Collins and Sunderland, 1974) for which most of the resulting plants were haploids Diploids, triploids, tetraploids, and hexaploids were reported by several authors In the cereals, a higher percentage of the resulting plants were albinos or green-albino chimeras Another difficulty in cereals is the low percentage of callus formation from anther culture that varied from 0 04% in maize (Murakami et al , 1972) to 32% in one barley genotype (Grunewaldt and Malepszy, 1975)

Knowledge of the anther stage at which haploid induction can take place is important Sunderland (1974) reported that this stage can be precisely defined and lies between the quartet stage and a stage just past the first pollen mitosis in those plants that have been investigated

The last method of haploid production mentioned here is chromosome elimination This method was first reported by Kasha and Kao (1970) It resulted from crossing cultivated barley, *Hordeum vulgare* ($2x=14$), with its wild relative *H bulbosum* ($2x=14$) Fertilization in this hybrid and subsequent mitotic elimination of the *H bulbosum* chromosomes in the developing embryo was observed by Subrahmanyam and Kasha (1973) Since Kasha and Kao's original report, several other successful attempts with this approach have been made The yield of haploids from this interspecific cross has steadily increased Kasha and Kao obtained 23 haploid seedlings from 209 cultured embryos (11 0%), while Jensen (Kasha,

Table 15.1 Cultivated species in which anther culture for haploid production has been applied

Species	Year	Authors
Tobacco	1967	Bourgin and Nitsch
	1968	Nitsch et al
	1969	Nitsch and Nitsch
Rice	1968	Nakata and Tanaka
	1969	Tanaka and Nakata
	1968 1971	Niizeki and Oono
Barley	1971 1973	Clapham
	1975	Grunewaldt and Malepszy
Tomato	1971 1972	Sharp et al
	1972	Gresshoff and Doy
	1973	Debergh and Nitsch
Asparagus	1972	Pelletier et al
Potato	1972	Irikura and Sakaguchi
	1973	*Dunwell and Sunderland*
Wheat	1973	Ouyang et al
	1973	C C Wang et al
	1973	Chu et al
	1973	Picard and Buyser
Triticale	1973	Y Y Wang et al
	1974	Sun et al
Eggplant	1973	Raina and Iyer
Pepper	1973	George and Narayanaswamy
	1973	Y Y Wang et al
	1973	Kuo et al
Pelargonium	1973	Abo El Nil and Hildebrandt

1974) reported 215 from 314 cultured embryos (68 5%) Apparently, the *H bulbosum* chromosome loss from the embryos in this procedure is gradual Subrahmanyam and Kasha (1973) found that 3 to 5 days after pollination, 40% of the dividing embryo cells were haploid but 11 days after pollination, 94% were haploid This chromosome elimination after wide hybridization in plants is similar to that reported from somatic cell hybridization in mammals (Weiss and Green, 1967 Allderdice et al , 1973) One of the suspected reasons for chromosome elimination is the difference in duration of the somatic cell cycles in the two parents involved (Gupta 1969 Lange, 1971, Subrahmanyam and Kasha, 1973) Apparently, the somatic cell cycle in *H bulbosum* is longer than in *H vulgare* (Barclay, Finch and Bennet personal communication Cited in Riley, 1974)

15 1 2 Meiotic Behavior of Monohaploids

As mentioned in Section 15 1 one has to distinguish between two major classes of haploids monohaploids and polyhaploids Monohaploids have only one basic genome (x) and consequently are meiotically very irregular In order to have meiosis functioning properly there must be two homologous chromosomes present for each chromosome type of the complement Meiosis in a number of monohaploids has been studied in sorghum (Schertz, 1963 Reddi 1968) rye (Heneen 1965) maize (Ting 1966 1969 Ford 1970 Weber and Alexander 1972) rice (Chu 1967) tomato (Ecochard et al 1969) pearl millet (Manga and Pantalu 1971) barley (Sadasivaiah and Kasha 1971 1973) and tobacco (Collins and Sadasivaiah 1972) Pairing of chromosomes in monohaploids (intragenomic pairing) has been thought to be the consequence of chromosome duplication and genetic redundancy Such pairing has been observed in rice tomato maize and barley It is interesting that even synaptonemal complexes have been observed under the electron microscope in studies of pachytene in monohaploids of tomato (Menzel and Price 1966) maize (Ting 1969 1971) and petunias and snapdragons (Sen 1970) Such complexes were similar in nature to those observed in the corresponding diploid forms In spite of these synaptonemal complexes often being formed in pachytene the chromosomes appear mostly as univalents in the diakinesis of these monohaploids However bivalents and multivalents were occasionally observed Ting (1966) found cells with one or more bivalents in 50 9% of all cells observed in maize Sadasivaiah and Kasha (1971 1973) even observed quadrivalent structures Metaphase I is striking in monohaploids in that the spindle is highly disorganized The chromosomes are mostly bivalents and trivalents also occur In anaphase I the distribution of the chromosomes to the opposite poles is usually at random Spindles seem to function weakly in some cases but the distribution mechanism is not yet very thoroughly studied

The pairing mechanism of nonhomologous chromosomes in the meiosis of monohaploids is not yet understood However Rieger (1957) had an interesting theory which states that all chromosomes have a certain tendency for pairing in meiotic prophase If homologous chromosomes are present in the cell then those are preferentially paired If such homologues are not present in the cell then the tendency to pair is satisfied by forces that can unite nonhomologous chromosome segments This reminds one of the precocity theory of Darlington (1932) mentioned in Chapter 1 which states that single chromosomes are in an unsatisfied or unsaturated state electrostatically and in order to become saturated they must pair

15 1 3 Meiotic Behavior of Polyhaploids

Since the meiosis of polyhaploids should be considered the nature of different kinds of polyploidy must be briefly mentioned here though this topic is going to be more fully discussed in Section 15 3 Polyploids can be either autoploid or alloploid, depending on their origin In true autoploids all basic genomes (x) have the same origin (e g AAAA) In alloploids basic genomes are of different origin (e g

AABB) Consequently polyhaploids can be classified into **autopolyhaploids** (e g , AA) or **allopolyhaploids** (e g , AB) (Kimber and Riley, 1963) Meiotic studies of polyhaploids may or may not help to determine which of these two types of poly haploidy are present in a given polyploid species If no pairing of chromosomes or chromosome segments takes place (e g , AB) the species under consideration is generally thought more likely to be an allopolyploid Pairing of chromosomes in a polyhaploid is indicative of some chromosome relationship either of **homoeologous** or of **homologous** nature Homoeology (Huskins, 1932) is partial homology during which some chromosome segments may pair and others do not pair In Section 15 1 2 it was mentioned that even in monohaploids, some chromosome pairing may occur (**intragenomic pairing**) However, since homologous chromosome pairing is thought to happen preferentially, the majority of pairing observed in polyhaploids probably is caused by partial or complete homology between genomes (**intergenomic pairing**). Most naturally occurring polyploids are of alloploid (e g , AABB) or **segmental alloploid** ($A_1A_1A_2A_2$) nature (Stebbins, 1950) Consequently, the majority of autopolyhaploids are derived from artificially induced autopolyploids Since this hypothesis is not generally accepted, the liter ature is full of proposed cases of natural autopolyploids and, consequently, autopolyhaploids that are derived from them (e g , Kimber and Riley, 1963) The author believes that most naturally derived polyploids are products of hybridiza tion (e g , A_1A_1 x A_2A_2 → A_1A_2) and subsequent chromosome doubling (A_1A_2 → $A_1A_1A_2A_2$) Since the races involved in the original hybridization (e g , A_1A_1 and A_2A_2) have generally grown in separate environments, they very likely have undergone different genetic and cytological changes (A → A_1, A → A_2) A high incidence of chromosome pairing associated with high seed fertility in polyhaploids of alfalfa (Stanford and Clement, 1955, Bingham and Gilles, 1971, Stanford et al 1972) and potato (Ivanovskaja, 1939), for instance, has led to the widespread conclusion that these cultivated species are autopolyploids However, the pairing of chromosomes is not entirely a measure of genome relationships, since this process is independently under a rigid genetic control (Riley and Law, 1965) In alfalfa Bingham and Gilles observed incomplete pairing (average $6^{II} + 4^{I}$) in one polyhaploid strain Ten of the 35 pollen mother cells had 6 or more univalents and one had 16 One other strain showed occasional bridges at anaphase I (indi cation for inversion during A → A_1 changes) These strains also showed varying degrees of female and male sterility indicating cryptic changes in the A_1 and A_2 genomes, which are not identifiable on the basis of abnormal meiosis In potato, Yeh et al (1964) *found from 16% to 26% cells with univalents in polyhaploids* Most of those present findings lead one to the conclusion that alfalfa and potato are also segmental allopolyploids ($A_1A_1A_2A_2$) rather than autopolyploids (AAAA)

The most important groups of allopolyhaploids, consequently, are segmental allopolyhaploids and allopolyhaploids Allopolyhaploids are obviously the least questionable group Absence of pairing in polyhaploids may be a valid means of con cluding allopolyploidy in the parents But under certain circumstances pairing can be induced between partially homologous chromosomes of allopolyhaploids This was detected by Riley (Riley and Chapman, 1958, Riley, 1960) in wheat, which

is considered to be an alloploid (*AABBDD*) Five different nullisomic 5B allopolyhaploids showed greatly increased chromosome pairing that apparently was caused by the absence of one or more genes that are responsible for the prevention of homoeologous pairing located in chromosome 5B A similar system was reported for oats (Gauthier and McGinnis, 1968) Limited pairing in allopolyhaploids was found in tobacco (Lammerts, 1934, Collins and Sadasivaiah, 1972), *Brassica* (Ramanujam and Srinivasachar, 1943) cotton (Barrow 1971), oats (Nishiyama and Tabata, 1964), the grass *Festuca arundinacea* (Malick and Tripathi 1970), and other species.

15 1 4 Possible Use of Haploids

The main reason for plant breeders to obtain haploids has been to develop a new and rapid method of breeding homozygous diploids or polyploids Since in a haploid every gene is presented only once, doubling of the chromosome should theoretically result in complete homozygosity. Repeated inbreeding for homozygosity in plants takes many generations, but doubling of haploids results in immediate homozygosity This doubling may occur naturally or may be induced by tissue wounding, heat treatment, colchicine or other chemical application, or decapitation A recent development is the possibility of obtaining homozygous diploids directly from anther cultures Niizeki and Oono (1971) reported that they had obtained diploid rice plants directly from pollen and not from somatic cells. Narayanaswamy and Chandy (1971) obtained 70% diploids, 23% triploids, and 7% haploids from anther cultures of haploid *Datura metel* Engvild (1974) received 20% diploids in tobacco when the anthers were cultured at the nuclear pollen stage.

In maize breeding, haploids obtained by natural parthenogenesis have been used with success in the production of homozygous diploid strains of commercial value (Yudin and Khvatova, 1966, Gyulavari, 1970, Petrov and Yudin, 1973)

In maize (Thompson, 1954) and in barley (Park et al., 1974), it has been established that the genetic variability for factors such as yield, heading date, plant height, etc., is the same for the monoploid method as for other conventional breeding techniques

In the Solanaceae, haploid plant breeding is well in progress Morrison (1932) reported the development of a diploid, commercial tomato strain from a haploid source. Similar results in tomato were reported by Kirillova (1965) Other work in progress is reported for pepper (Y Y Wang et al., 1973) and some tobacco species (Nöth and Abel, 1971, Nitsch, 1972)

15.2 Diploidy

Just as the term *haploidy* is used in a general and a specific way, the term *diploidy* has also two different meanings In its general sense it designates organisms with two homologous chromosome sets Each type of chromosomes is always represented twice. Sex chromosomes are exceptions In this wider sense even polyploids

could be designated as **functionally diploid** (2n) On the other hand, the term diploidy is used more specifically as a distinction between monohaploids (x), diploids (2x) and polyploids (3x, 4x, etc)

15 2 1 Diploidization

The term "*diploidization*" describes a selection process that causes polyploids that are originally meiotically irregular, having multivalents and bridges, to become meiotically regular like ' good diploids " The end product of such a process if often called an **amphidiploid**, which is a diploid like polyploid or a functional diploid, as described in Section 15 2

15.3 Polyploidy

Polyploid individuals or populations have more than two basic genomes or chromosome sets (3x, 4x, 6x, etc) They are particularly prominent in the plant kingdom Among the angiosperms, 30% to 35% of the species are polyploid (Darlington and Janaki-Ammal, 1945, Stebbins, 1950) Almost 75% of the Gramineae are polyploid Polyploidy is thought to be a product of interspecific hybridization Polyploids occupy different niches than their related diploids It seems that polyploids possess a wider ecological range of tolerances They often can occupy habitats that cannot be occupied by diploids (Swanson, 1957)

15 3 1 Classification of Polyploidy

For the classification of polyploids, the use of **genome formulas** is a handy tool In the genome formula a capital letter represents a group of chromosomes that is generally referred to as the basic genome or chromosome set Such a chromosome set corresponds to the basic chromosome number that, for instance, in the Gramineae is 7 or 10 Many species of Gramineae have multiples of 7 chromosomes The genome formula for wheat, for instance, is AABBDD The letter "A" represents a basic genome of 7 chromsomes

For the characterization of different types of polyploids, the best representation is still the one by Stebbins (1950) (Fig 15 1) According to this scheme there are four major types of polyploids recognizable

1 Autopolyploids = AAAA
2 Segmental Allopolyploids = $A_1A_1A_2A_2$
3 Genome Allopolyploids = AABB
4 Autoallopolyploids = AABBBB

Autopolyploids have chromosome sets (A) or basic genomes in which the chromosomes are entirely homologous to each other, which results in complete pairing in meiosis Terms like **autotriploidy, autotetraploidy, autopentaploidy,** and **autohexaploidy** indicated that there exist three, four, five, and six homologous basic genomes per cell

Segmental allopolyploids (Stebbins, 1947b) are characterized by **homoeology** (Huskins, 1932) or **partial homology**, also called **residual homology** by Stephens

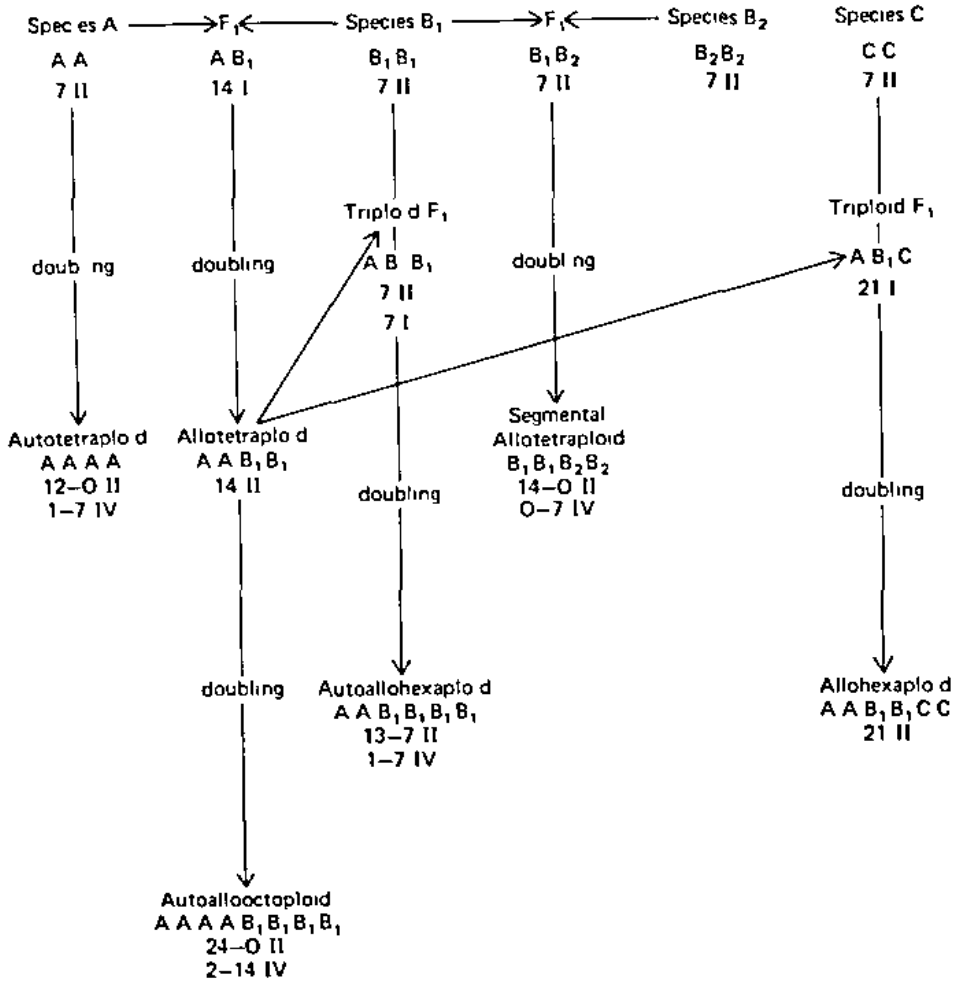


Fig 15 1 Diagram illustrating genome relationships between autopolyploids, allopolyploids, segmental allopolyploids, and autoallopolyploids (From Elliott, 1958 Modified from Stebbins, 1950 Redrawn by permission of Columbia University Press, New York)

(1942) This type of homology may indicate that only some of the members of the chromosome set are homologous with those of the other set or sets, while the others are nonhomologous or only partially homologous This kind of polyploidy includes a wide array of types that ranges all the way from nearly autoploid to the other extreme, almost alloploid

Genome allopolyploids are believed to be derived by hybridization of parents that *had striking structural dissimilarity between their basic genomes Chromosomes* in meiosis are limited to bivalent pairing Genome allopolyploids received their name because the entire genome, for instance, of parental species A is different from that of parental species B, forming after hybridization and doubling a new functional diploid with two new AB sets (AABB) Genome allopolyploids also can occur at different ploidy levels and can produce allotriploids (AAB), allotetraploids (AABB), allopentaploids (AABBC), allohexaploids (AABBCC), etc

Crucial for further classification of these alloploids is the basic chromosome number of the contributing diploid parents Darlington and Janaki-Ammal (1945) dis-

tinguished between di , tri , or **polybasic polyploids.** Typical examples for dibasic polyploids are brown mustard *(Brassica juncea)* rape *(B napus)* and *B carinata* (Fig 15 2) These three *Brassica* species are believed to have derived from the three basic genomes of black mustard(*B nigra* = N) (*B campestris* = C) and cabbage (*B oleracea* = O) The basic chromosome numbers are

B nigra x = 8 (N)
B oleracea x = 9 (O)
B campestris x = 10 (C)

The genome formulas for the dibasic polyploids are

B juncea = NNCC (2n = 36)
B napus = CCOO (2n = 38)
B carinata = OONN (2n = 34)

However many alloploids are **monobasics** in which the contributing diploid parents have identical basic chromosome numbers

15 3 2 Autopolyploidy

There have been different opinions about the frequency of autopolyploids in nature This stems in part from the different interpretation of what actually is an autoploid For instance Muntzing (1936) included segmental allopolyploids in the autoploid category and stated that autopolyploidy is quite common in plants However Stebbins (1950) believed that autoploids are relatively rare in nature As a matter of fact the only clear-cut case of autoploidy in nature according to Stebbins is *Galex aphylla* which was reported by Baldwin (1941) According to Stebbins several cultivated crops can be classified as being either autoploid (segmental alloploid inclusive) or alloploid (Table 15 2)

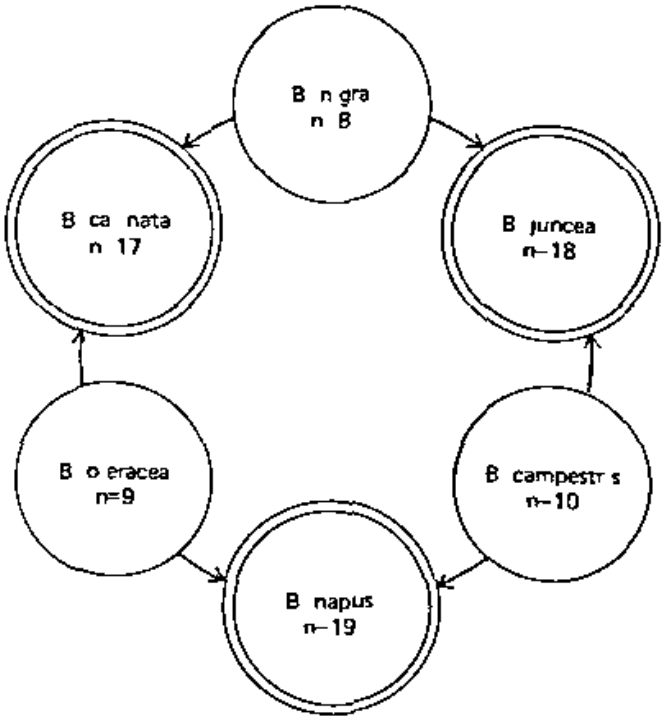


Fig 15 2 The origin of polybasic polyploids in the genus *Brassica* (After Morinaga 1934 and U 1935 Redrawn by permission of McGraw Hill Book Company Inc New York)

Table 15 2 Crop plants listed according to their classification as autoploids (segmental alloploids respectively) or alloploids (modified from Elliott, 1958)

Common name	Scientific name	x number	2n-number
A *Autoploids*			
(incl segmental alloploids)			
Potato	*Solanum tuberosum*	12	48
Coffee	*Coffea arabica*	11	22,44 66 88
Banana	*Musa sapientum*	11	22,33
Alfalfa	*Medicago sativa*	8	32
Peanut	*Arachis hypogea*	10	40
Sweet potato	*Ipomea batatas*	15	90
B *Alloploids*			
Tobacco	*Nicotiana tabacum*	12	48
Cotton	*Gossypium hirsutum*	13	52
Wheat	*Triticum sativum*	7	42
Oats	*Avena sativa*	7	42
Sugar cane	*Saccharum officinarum*	10	80
Plum	*Prunus* spp	8	16 24 32 48
Loganberry	*Rubus loganobaccus*	7	42
Strawberry	*Fragaria grandiflora*	7	56
Apple	*Malus sylvestris*	17	34 51
Pear	*Pirus communis*	17	34,51

Phenotypically, autoploids are generally larger in size than their diploid counterparts, but there can be exceptions to this The cytoplasm and the nucleus of autoploids are larger than those of diploids, this will lead to giant growth characteristics, provided the cell number also increases proportionally Very often, however, the cell number does not match with those of the diploids, particularly in artificially produced autoploids

15.3.2.1 Autoploidy in Plant Breeding Several methods have been applied to produce polyploids in cultivated crops A few should be mentioned here (Briggs and Knowles, 1967)

1 decapitation
2 indoleacetic acid
3 *twin seedlings*
4 heat treatments
5 colchicine
6 other chemicals

Decapitation and generation of callus tissue with or without the use of indoleacetic acid to stimulate regrowth has been successfully used in *Solanum* and *Nicotiana* The production of twin seedlings was one of the earliest methods of obtaining polyploids Twin embryos are occasionally found in a low frequency among germinating seedlings and often yield heteroploid plants Heteroploidy (Winkler, 1916) is the phenomenon that shows deviation from the normal chromosome number The twin seedling method was already mentioned during the discussion of haploid production (Section 15 1 1) Müntzing (1937) was one of the first to recognize that twin seedlings could also be autoploids High temperature treatments for short periods have been employed for the production of polyploids in plants such as maize (Randolph, 1932)

Table 15 3 Cultivated crops that were developed from artificial and spontaneous autoploids

Common name	Scientific name	Basic chrom no (x)	Normal chrom no (2n)	Autoploid chrom no (2n)	References
Maize	*Zea mays*	10	20	40	Randolph, 1932
Rye	*Secale cereale*	7	14	28	Müntzing 1951, 1954
Alsike clover	*Trifolium hybridum*	8	16	32	Levan, 1942a
Red clover	*Trifolium pratense*	7	14	28	Levan, 1942a
Sugar beet	*Beta vulgaris*	9	18	27,36	Schlösser, 1936, Frandsen, 1945, Hagberg and Åkerberg, 1962
Turnip	*Brassica campestris*	10	20	40	Elliott, 1958
Banana	*Musa paradisiaca*	11	22	33,44	Dodds and Simmonds, 1938
Apple	*Malus sylvestris*	17	34	51	Einset and Lamb, 1951
Narcissus	*Narcissus bulbocodium*	7	14	21,28,35,42	Fernandes 1934
Garden tulip	*Tulipa gesneriana*	12	24	36	Upcott and LaCour, 1936
Garden hyacinth	*Hyacinthus orientale*	8	16	24,32	Darlington et al , 1951
Gladiolus	*Gladiolus* spp	15	30	45,60,75 90, 120	Bamford, 1935
Garden dahlia	*Dahlia variabilis*	8	32	64	Lawrence, 1931a
Florists' cyclamen	*Cyclamen persicum*	12	48	96	De Haan and Doorenbos, 1951
Snapdragon	*Antirrhinum majus*	8	16	32	Stebbins, 1957
Easter lily	*Lilium longiflorum*	12	24	48	Emsweller, 1951
Watermelon	*Citrullus vulgaris*	11	22	33	Kihara, 1951b
Common day lily	*Hemerocallis fulva*	11	22	33	Mookerjea, 1956
Grape	*Vitis vinifera*	19	38	76	Olmo, 1952
Orchid	*Paphiopedilum insigne*	13	26	39	Mehlquist, 1947
Forsythia	*Forsythia intermedia spectabilis*	14	28	42,56	Hyde, 1951
Iris	*Iris mesopotamica*	12	24	48	Sturtevant and Randolph, 1945
Perennial ryegrass	*Lolium perenne*	7	14	28	Myers 1939, Sullivan and Myers, 1939

Fig 15 3A and B Comparison between (*A*) diploid (2n = 16) and (*B*) tetraploid (2n = 32) snapdragons (Courtesy of W Atlee Burpee Co , Doylestown PA)

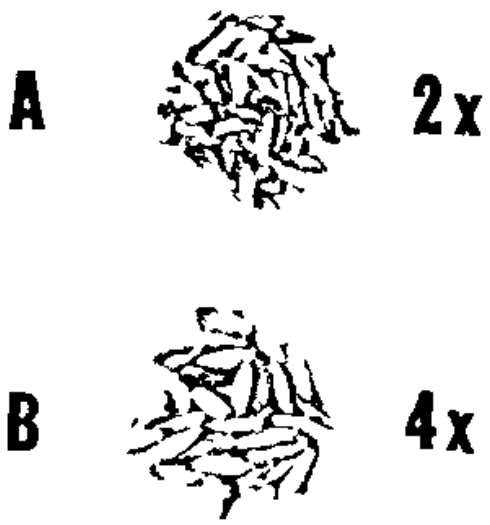


Fig 15 4A and B Comparison of seeds and spikes of (*A*) diploid (2n = 2x = 14) and (*B*) tetraploid (2n = 4x = 28) rye (Courtesy of Dr Herb Luke Department of Plant Pathology University of Florida Gainsville)

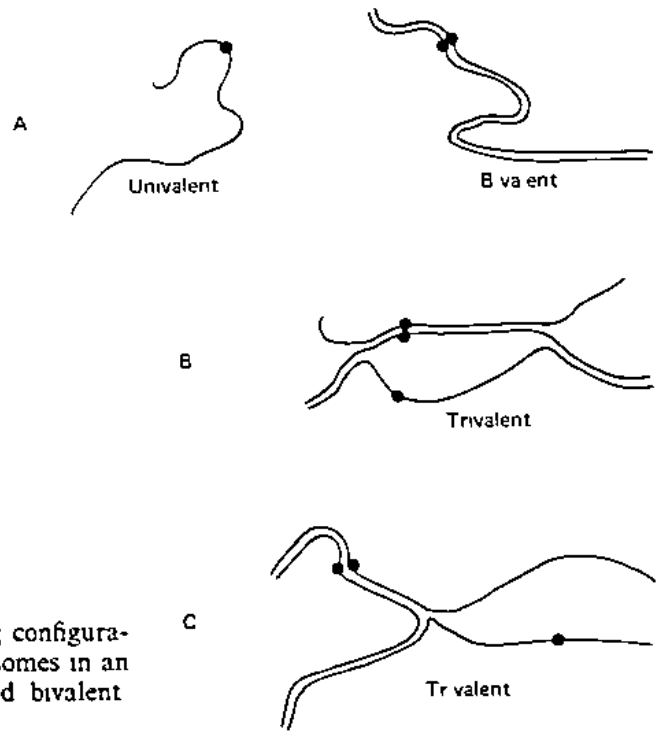


Fig 15 5A C Possible pairing configurations of 3 homologous chromosomes in an autotriplod (*A*) Univalent and bivalent (*B C*) Trivalents

present The other requirement is chiasma formation in the paired segments If no chiasma is formed in a paired region, the homologous chromosomes separate in that region during diplotene Four different possible trivalent configurations in autotriploids are shown in Fig 15 6A

The region where one chromosome (e g , chromosome 1) changes its pairing association from one pairing partner (e g , chromosome 2) to another (e g , chromosome 3) is called the point of **partner exchange** (Darlington, 1929b) It has been observed that there is a reduction of chiasma frequency around the point of partner exchange (Sybenga, 1975)

Chromosome separation in meiosis I from trivalents is irregular Daughter nuclei will receive either one or two chromosomes from any given trivalent Therefore, each sister nucleus will have a haploid set of chromosomes plus additional ones. Consequently most of the gametes resulting from autotriploid individuals do not have balanced chromosome complements and are not viable If progeny survives from triploids it is mostly **aneuploid** (Chapter 16)

The fact that high sterility results from triploids has been explored in polyploid breeding Triploid bananas (2n = 33) are vigorous but seedless and are therefore preferred for food consumption Likewise, triploid watermelons, which were developed in Japan, have undeveloped seeds that naturally are of great advantage Such seeds are no more objectionable than those in cucumbers (Kihara, 1951b) (Fig 15 7) Triploid offspring (3x) has been produced from 2x x 2x interspecific *Citrus* crosses caused by fertilization of unreduced egg cells This method could be utilized for breeding seedless *Citrus* cultivars (Geraci et al , 1975)

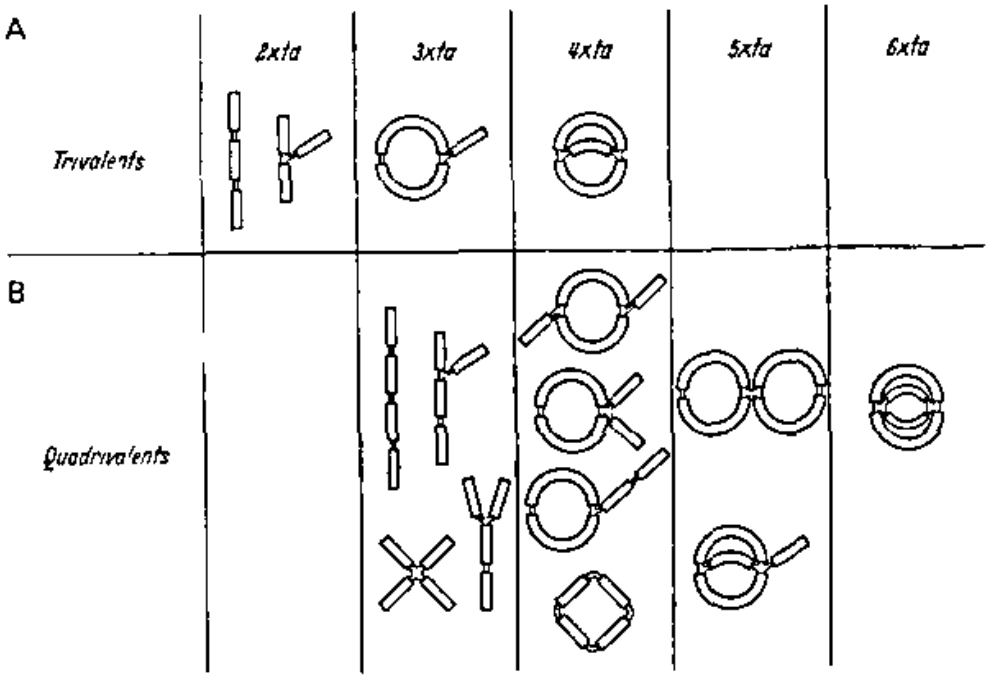


Fig 15 6A and B Different possible types of trivalents (*A*) and quadrivalents (*B*) in meiosis I (xta = chiasmata) (From Rieger et al , 1976)

According to Kuliev et al , (1975), triploidy in humans was the most frequent karyotype anomaly in a cytogenetic study of more than 4000 abortuses A total of 323, about 8 1%, fetuses were found to be triploid This is in close agreement with data from Jacobs et al (1978), who found 28, or 8 2%, triploids among 340 spontaneous abortions In a review of triploidy in humans, Niebuhr (1974) reported clinical data on about 230 triploid abortuses and 33 live-born triploid infants Triploids surviving for more than a few days were all diploid triploid mosaics
One was a 3-year-old boy with micrognathia (small skull), syndactyly (grown together fingers or toes) and mental retardation Blood cultures were almost completely diploid, but in primary skin cultures, about 92% of the cells were triploid and 8% diploid (Böök and Santesson, 1960, Böök et al , 1962) Other malformations observed in human triploids are hydrocephalus or relatively large heads, malformed ears and eyes, and cleft palate Jacobs and co-workers calculated that 66 4% of all human triploids were the result of **dispermy** (see Chapter 1, Boveri), 23 6% the result of fertilization of a haploid ovary by a diploid sperm, and 10% the result of a diploid egg fertilized by a haploid sperm

15 3 2 3 Autotetraploids In autotetraploids there exist four homologous sets of chromosomes Theoretically, the affinity between the four homologous chromosomes is equal If one would assume only one single point of chromosome pairing initiation, there would be only a chance for bivalent formation since pairing is in two-by-two fashion If there would be two such initiation points, then partner exchange could occur, and a chance for **quadrivalent** formation would exist Just as in triploids, the kind of pairing configurations will be determined by the kind of pairing and the number and location of the chiasmata formed Examples of the

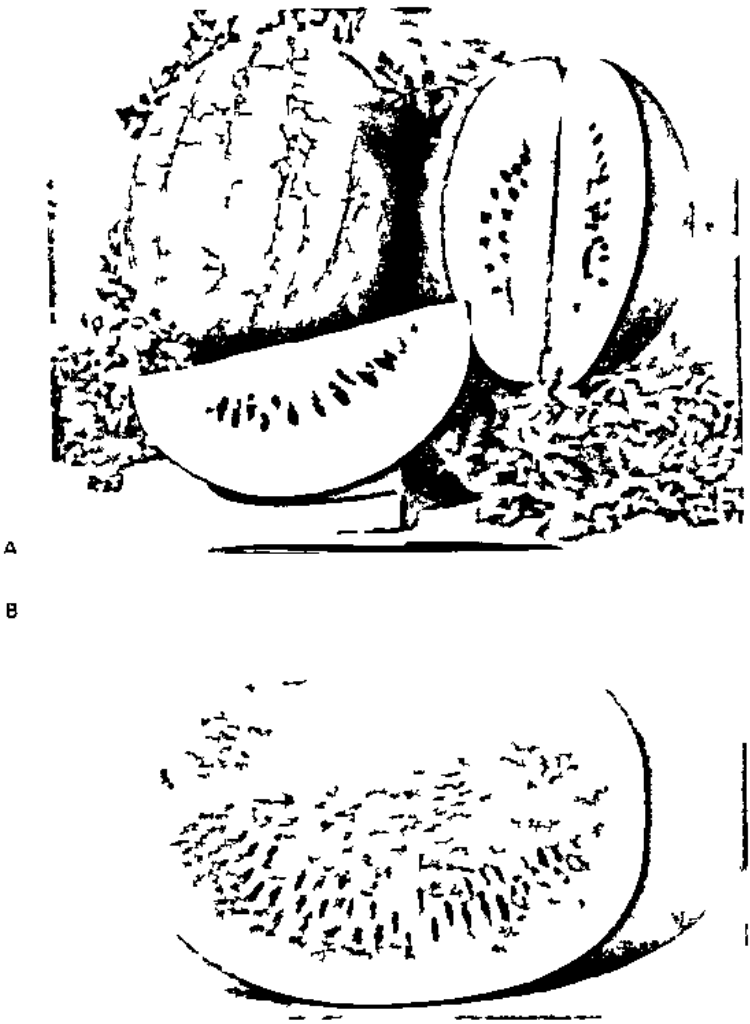


Fig 15 7A and B (*A*) Diploid watermelon (2n = 2x = 22) with numerous seeds (*B*) Trip loid (2n = 3x = 33) variety with few and imperfect seeds (Courtesy of W Atlee Burpee Co Doylestown PA)

possible shapes of quadrivalents in diakinesis are shown in Fig 15 6B That figure shows that there are 11 different shapes possible for quadrivalents Any other combinations of univalents bivalents and trivalents add to the possible expression of pairing in meiosis I According to quadrivalent analysis the 11 configurations shown in Figure 15 6B do not occur at random For instance in tomato and peren nial ryegrass more than 50% of the configurations are rings of four homologous chromosomes and about 30% are chains (Dawson 1962) Most autotetraploids have an abundance of these two kinds of quadrivalents The arrangement of these multivalents in metaphase is similar to that of those discussed under transloca tions (Chapter 14) If all centromeres are oriented toward the poles and attached by the spindle fibers one speaks of **coorientation** If only two of the four centro-meres are oriented toward the poles (**noncoorientation**) **false univalents** (Upcott

The gamete ratio in this instance is $1AA\ 4Aa\ 1aa$ assuming that the two sister chromatids of a chromosome always move into different gametes (chromosome segregation) Relative genotype frequencies can be calculated from such gametic ratios If a duplex individual ($AAaa$) is selfed, one can expect the following offspring

Gametes	$1AA$	$4Aa$	$1aa$
$1AA$	$1AAAA$	$4AAAa$	$1AAaa$
$4Aa$	$4AAAa$	$16AAaa$	$4Aaaa$
$1aa$	$1AAaa$	$4Aaaa$	$1aaaa$

According to this **Punnett square**, the phenotype frequencies in a strictly complete dominant fashion are 35 dominants to one recessive (35 1) However, in actual experimental data this ratio is usually not that high but approaches more a ratio of $21A\ 1a$ As mentioned, crossing over between the gene and the centromere disturbs the typical tetrasomic ratio and causes a phenomenon called **double reduction** (Fig 15 8) This phenomenon causes an increase in recessive gametes It occurs when at the end of meiosis the two sister chromatids of a chromosome end up in the same gamete Figure 15 8 demonstrates double reduction in a duplex ($AAaa$) The chromatids carry the genes $a_1a_1/A_3A_3/a_2a_2/A_4A_4$ on four homologous chromosomes that are separated by dashes This is the situation before crossing over After crossing over the four homologous chromosomes carry the genes $a_1A_3/a_1A_3/a_2A_4/a_2A_4$ Without double reduction (Fig 15 8A) all gametes are dominant ($4Aa.Oaa$) With double reduction in two of the four gametes (Fig 15 8B) the gamete ratio is three dominants to one recessive ($1AA\ 2Aa\ 1aa$) With double reduction in all four gametes (Fig 15 8C), the gamete ratio is one dominant to one recessive ($2AA\ 2aa$) As indicated with increasing double reduction the number of recessives rises

If random chromosome assortment does not occur, as in tetrasomic inheritance, the gamete ratio decreases from 5 1 ($1AA\ 4Aa\ 1aa$) to 3 1 ($1AA\ 2Aa\ 1aa$) If the genes on the two chromosome pairs (1 2) are designed A_1a_1/A_2a_2 and only A_1 can pair with (and segregate from) a_1 and not with A_2 and a_2, then the following gametes are produced

A_1	A_2	a_1	a_2		Gametes
*	*				$1\ AA$
*			*	}	$2\ Aa$
	*	*			
		*	*		$1\ aa$

Since only A_1 carrying chromosomes segregate from A_1's, and A_2's from A_2's, only A_1 and A_2 carrying chromosomes can end up in the same gametes and not A_1's and A_1's

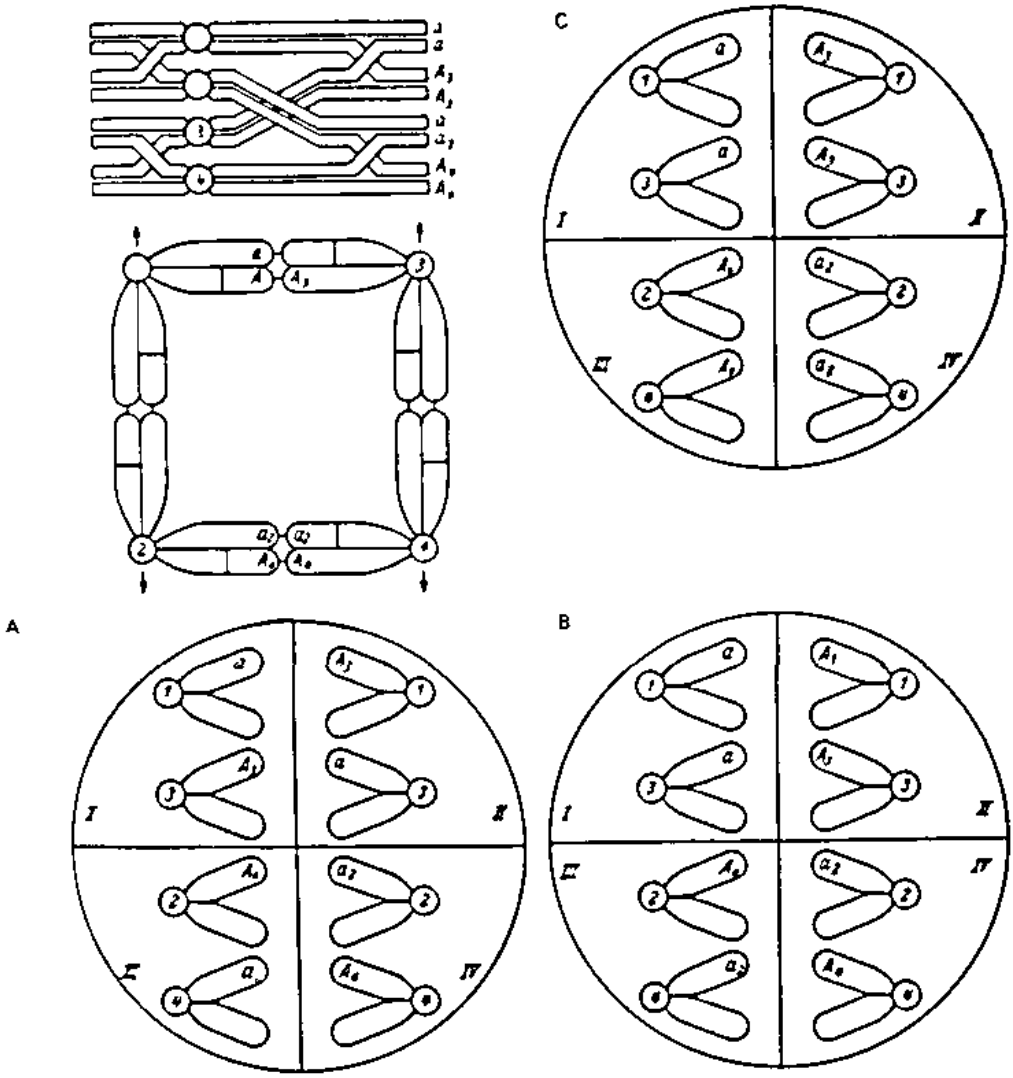


Fig. 158A, B and C Schematic representation of double reduction in an autotetraploid (*A*) No double reduction (*B*) Double reduction in two of four gametes (*C*) Double reduction in all four gametes (From Rieger and Michaelis 1958)

If an individual with such gametes is selfed the following progeny results

Gametes	1*AA*	2*Aa*	1*aa*
1*AA*	1*AAAA*	2*AAAa*	1*AAaa*
2*Aa*	2*AAAa*	4*AAaa*	2*Aaaa*
1*aa*	1*AAaa*	2*Aaaa*	1*aaaa*

A typical dihybrid ratio of 15*A* 1*a* is the result This expression is also called **duplicate gene expression** This phenomenon is caused by two identical allele pairs (*A A A A*$_2$) that have the same phenotypic expression but are located on two different chromosome pairs

one speaks of wide hybridization In wide hybridization one often encounters the phenomenon of **chromosome elimination** or **chromosome diminution.** This phenomenon has also been called **Rückregulierung** (Rieger and Michaelis, 1958) or downward adjustment, which is the tendency of polyploid or mixoploid tissues to return to the original chromosome number of one of the diploid parents The mechanism of this adjustment is not very well known yet It is suspected that one of the ways to accomplish this is the formation of multipolar spindles with subsequent elimination of occurring unbalanced cells (Rieger and Michaelis, 1958) In autoploids this cytological instability is not very frequent For instance, in the progeny of artificially produced tetraploid barley the frequency of diploid individuals is only 1 in 5000 to 6000 (Müntzing, 1957) If, however, genomes of very remote species are combined by **somatic cell hybridization** or **cell fusion** (see Chapter 1, Harris and Watkins, 1965) then chromosomes of one of the parents are usually successively eliminated in the alloploid cell cultures (Ephrussi and Weiss, 1969, Zepp et al , 1971) In human-mouse somatic cell hybrid cultures Weiss and Green (1967) observed that after 100 to 150 generations only two or three human chromosomes remained

Most alloploid species are tetraploid Hexaploid alloploids are less common It is still very difficult to obtain ploidy levels higher than octoploid experimentally Examples of very high ploidy levels in nature are given in Table 15 6

Much attention has been directed to an intergeneric alloploid combination between wheat and rye, called *Triticale* The first fertile *Triticale* hybrid was achieved by the German private plant breeder Wilhelm Rimpau in 1888 (Jenkins, 1969) Since then many attempts were made to develop a new crop from such a combination The hope is to combine into one plant type the nutritional and baking qualities of wheat with the drought tolerance, adaptation to poor soils, and disease resistance of rye Early *Triticale* breeding programs were based on octoploid combinations such as *AABBDDRR in which three wheat genomes (ABD)* were combined with the rye genome (R) However, it later was discovered that the hexaploid combinations such as AABBRR were more successful The most

Table 15 6 Natural and artificial alloploids with very high chromosome numbers

Species	Basic no (x)	Somatic no (2n)	Ploidy level	Reference
Hisbiscus radiatus x *H diversifolius*	9	216	24 x	Menzel and Wilson, 1963
Galium grande	11	±220	20 x	Dempster and Stebbins 1968
Poa litorosa	7	265	38 x	Hair and Benzenberg 1961
Kalanchoe spp	17,18	500	30 x	Baldwin, 1938
Aulacantha	—	1000 2700	—	Grell and Ruthmann, 1964
Schizaea dichotoma	—	1080	—	Brown 1972
Ophioglossum reticulatum	—	1260	—	Ninan, 1958

promising hexaploid Triticales are the so-called secondary types that have been obtained from intercrosses between hexaploid and octoploid Triticales (Pissarev, 1963, Kiss, 1966) and between hexaploid Triticales and hexaploid wheats (Nikajima and Zennyozi, 1966, Larter et al., 1968, Jenkins, 1969, Zillinsky and Borlaug, 1971) Some of the agronomically interesting hexaploid Triticales are not Triticales in the real sense of this term but are more properly considered to be substitutional hexaploid Triticales since some of their R-genome chromosomes are substituted by D genome chromosomes (Gustafson and Qualset, 1974) However, the highest yielding fertile lines all appear to have seven pairs of rye chromosomes (Gustafson and Qualset, 1975)

Hexaploid combinations of wheat with *Agropyron* are being developed that could combine all the useful agronomic characteristics of durum wheat (AABB) with the vegetative vigor, disease resistance and winter-hardiness of *Agropyron* (Schulz-Schaeffer and McNeal, 1977) A very extensive program of wide hybridization among the grasses of the tribe Triticeae has been carried out by Dewey (1965, 1970, 1975) One of the goals of this program is to synthesize new amphiploid species and to evaluate their breeding potential (Asay, 1977) Dewey's work is also a practical application of Kihara's (1930) principle of genome analysis, which was discussed in the first chapter The genome relationships in the genera *Elymus*, *Agropyron*, and *Hordeum*, as worked out by this analysis, are shown in Fig 15 11

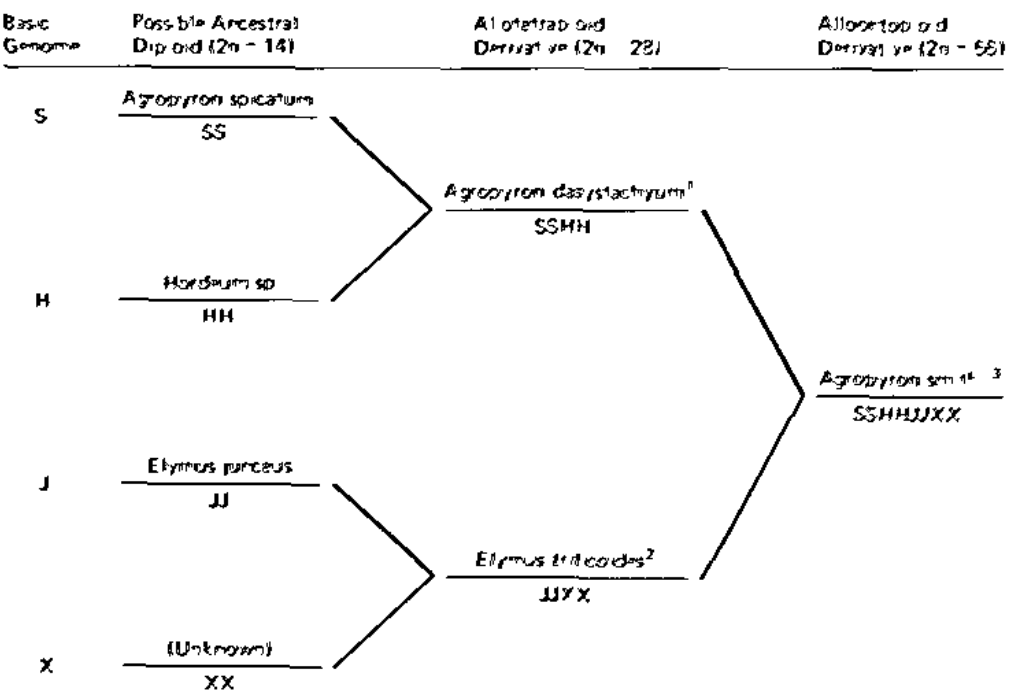


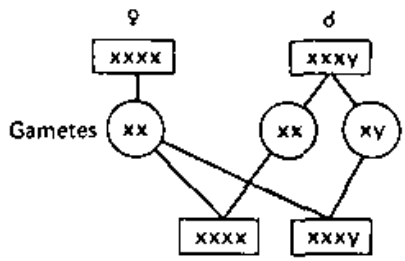
Fig 15 11 Species relationships in the tribe Triticeae as determined by genome analysis ([1]Dewey, 1965 [2]Dewey, 1970 [3]Dewey, 1975 Courtesy of Dr Douglas Dewey, Crops Research Laboratory, USDA, Utah State University, Logan)

15 3 5 Complications with Polyploidy in Man and Animals

It was mentioned in the preceding sections that polyploidy in man is extremely detrimental Three major reasons for the lack of polyploidy in animals have been mentioned (White, 1973)

1 disturbance of sex determining mechanism
2 cross fertilization barrier
3 histological barrier

Muller (1925) proposed that the reason for the paucity of polyploidy in animals compared with plants is that in bisexual forms, polyploidy could upset the sex chromosome mechanism If the diploid mechanism is XY XX the tetraploid one consequently should be XXYY XXXX It could be argued that during meiosis of the male the two Y chromosomes would pair and also the two X chromosomes with the result that only XY gametes would form If such a male gamete would fertilize a female egg (XX) only XXXY progeny would result and consequently only one sex This idea was voiced during a period when Bridges' Theory (Section 5 1 1) of balance between X chromosomes and autosomes based on *Drosophila* was accepted for animals in general But Muller's argument did not hold ground when it was established that in bisexual plants *(Melandrium album)* and in some animal groups (Urodeles and mammals) the control mechanism of sex differentiation is the presence or absence of the Y chromosome XXXY male individuals can backcross to XXXX females in the following fashion



Bogart and Tandy (1976) state that the prior assumption that bisexual polyploid animals are not able to overcome sexual imbalances in gametogenesis is not true anymore because of the recent discovery of several bisexual natural polyploid animal populations They found diploid and tetraploid populations of African anuran frogs included in the same bisexual species and thought they had established the fact that polyploidy is a general phenomenon in frogs and may appear in any genus

The second barrier cross fertilization, is closely linked to the necessity of bisexuality in animals With the low incidence of a tetraploid in a diploid population, the simultaneous occurrence of another tetraploid individual as mating partner is unlikely If such a tetraploid individual mates with a diploid it will produce sterile triploid progeny The high proportion of triploids among chromosomally abnormal aborted human fetuses verifies this assumption (Hamerton, 1971b)

The third barrier, caused by histological complications, is explained by the more complex nature of animals It is thought that polyploidy interferes with the devel-

opmental pattern of animals during tissue differentiation Fankhauser (1945) gave evidence for such complications in amphibians The size and the number of cells in such vital tissues as the brain, the spinal cord, and the nervous system are smaller than those of diploids A strong argument against the possible effectiveness of such a histological barrier is the high frequency of possible polyploidy in parthenogenetic animals (White, 1973)

A different phenomenon is the quite regular occurrence of specialized polyploid tissues in otherwise diploid animals Rat liver for instance, has 5% octoploid, 40% tetraploid, and 55% diploid cells (Dawson, 1962) This phenomenon is also referred to as **mixoploidy** (Nemec, 1910) or **chromosome mosaicism** (Section 17 1 3) The rectal glands of *Drosophila* are predominantly octoploid

Chapter 16
Aneuploidy

The previous chapter dealt with the effect of genomic changes in the number of chromosomes in living organisms In this context we can speak of a phenomenon of **genome balance** that preserves certain requirements of function It has been observed that loss or gain of one or more chromosomes may influence the meiotic pairing ability of chromosomes within balanced genome sets (Person, 1956, Tsuchiya, 1959, 1960, Schulz-Schaeffer et al , 1973) Genome imbalance is not only expressed as meiotic disturbance but also morphologically A change in the relative proportion of different genes has a phenotypic effect If a chromosome is added to or missing from a normal genomic multiple, this change is often visible in the organism If a whole genome is added the polyploid is often indistinguishable from the diploid form For instance, there is little difference between the diploid and tetraploid forms of *Galex aphylla* both of which exist in nature (Baldwin, 1941) In this chapter the effect of chromosome number changes that are not genomic in nature is being studied

16.1 Euploidy

Euploidy is the term for cells, tissues and individuals that have either the basic chromosome number of a genus (x) or complete multiples thereof (2x, 3x, 4x, etc) Some genera have more than one basic chromosome number and are recognized for this fact Examples are the genera *Crepis* (x = 4, 5, 6, 8), *Carex* (x = 6, 7, 8, 9, 10, 13, etc) *Brassica* (x = 8, 9, 10, 11), and *Viola* (x = 6, 10, 11, 13) In these genera it is more difficult to distinguish euploidy from **aneuploidy,** which deviates from euploidy in that it has incomplete multiples of the x-number Euploidy is often necessary for survival For instance, some euploid diploids can not afford to lose single chromosomes Monosomics of barley do not survive Every chromosome is necessary for the genome balance However, monosomics in maize have been isolated Haploid barleys have been reported and are viable (Clavier and Cauderon, 1951, Suzuki, 1959, Tsuchiya, 1962, Fedak, 1972, Kasha, 1974) In polyploids, aneuploidy is a common phenomenon and often goes unnoticed phenotypically The extra genomes function as genetic buffers in such a polyploid system

16.2 Aneuploidy

As already indicated, aneuploidy is any deviation from a euploid condition. This condition can be expressed either as an addition of *one or more entire chromosomes* or chromosome segments to a genomic number (1x, 2x, 4x, etc.) or as a loss of such chromosome material.

Aneuploidy can be caused by any of the four following disturbances (Rieger et al. 1976):

1. Loss of chromosomes in mitotic or meiotic cells, often caused by lagging chromosomes or **laggards**, which are characterized by retarded movement during anaphase. This results in **hypoploid** chromosome numbers (e.g., 4x − 1, 4x − 2, 2x − 1, 2x − 2, etc.).
2. Non disjunction of chromosomes or chromatids during mitosis or meiosis. This is a failure of such genetic units to separate properly and results in their not being distributed to opposite cell poles (Fig. 16.1). It can cause **hypo-** or **hyperploid** chromosome numbers (e.g., 4x − 1, 4x + 1, etc.).
3. Irregularities of chromosome distribution during the meiosis of polyploids with uneven numbers of basic genomes such as in triploids, pentaploids, etc. (e.g., 3x, 5x). In such polyploids, some chromosomes are often present as univalents. They are distributed randomly to either pole or may be lost in anaphase I or anaphase II.
4. The occurrence of multipolar mitosis, resulting in irregular chromosome distribution in anaphase. Such **multiform aneuploids** (Bělař, 1945) can result in cells with different aneuploid chromosome numbers, causing the formation of tissue with chromosome mosaicism.

16.2.1 Nullisomy

Hypoploids are individuals, tissues, or cells that are deficient for one or more chromosomes. One class of hypoploids are the nullisomics, which have one or more pairs of homologous chromosomes missing. Nullisomics usually are not found in natural populations but have to be obtained by intercrossing or selfing of monosomics (e.g., 6x − 1). This can occur by the fusion of two gametes that are lacking the same chromosome. Selfed monosomics produce disomic, monosomic, and nullisomic progeny. Since male gametes lacking a chromosome usually have a low survival rate during fertilization or are less competitive, the percentage of nullisomics from monosomic selfing is quite low. In wheat only from 0% to 10% of the 20-chromosome male gametes can compete with 21-chromosome male gametes during pollen tube growth. Therefore, only a small percentage of the progeny of selfed monosomics are nullisomic. Sears (1953) reported that monosomic 3B yields up

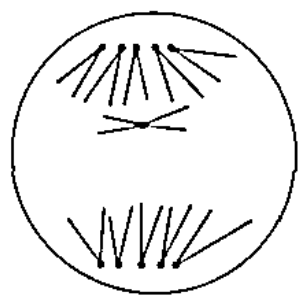


Fig. 16.1 Nondisjunction of chromatids during mitosis

to 10% nullisomics, and several other monosomics yield as little as 1% after selfing. Nullisomics are generally weak individuals that are difficult to maintain. They are reduced in fertility, size, and vigor. In wheat all 21 possible nullisomics (the **nullisomic series**) have been obtained by Sears. Only nullisomic strains 7B and 7D can be maintained easily as nullisomic stocks. Nullisomic series are not of great agronomic importance, but they can be used for genetic studies. The wheat nullisomics differ from each other morphologically and thus demonstrate the genetic effect of the missing chromosome pair (Fig. 16.2). **Nullisomic analysis** can be used to assign dominant genes to specific chromosomes (Dawson, 1962). The **disomic**[1] individual that shows a certain homozygous dominant character (e.g., *A*) can be crossed with a nullisomic series the members of which all show the recessive character. The offspring of these crosses will all be heterozygous (*Aa*) or **hemizygous**[2] dominants (*A*0). If the heterozygotes (*Aa*) are selfed, they will segregate 3*A* to 1*a*. The hemizygous monosomics (*A*0) will, upon selfing, result in a majority of dominants (*AA*+*A*0) and a small proportion with the recessive character being nullisomic. The nullisomic that upon crossing produces hemizygous F_1's and a ratio deviating from the normal 3:1 in the F_2 is the one that designates the carrier of the dominant gene (*A*) in question (Fig. 16.3).

16.2.2 Monosomy

As mentioned, monosomics are organisms with one missing chromosome (6x−1, 4x−1, etc.) (Fig. 16.4). Monosomics have been discovered in humans, animals, and plants. Three types of monosomy can be recognized (symbols show wheat situation):

1. *Primary Monosomy.* One chromosome is missing. The remaining homologue to the missing chromosome is a structurally normal chromosome. Rieger et al. (1976) also recognize this term (symbol 20″+1′ 2n=41).
2. *Secondary Monosomy.* One homologous chromosome pair is missing and is replaced by a **secondary chromosome** or isochromosome for one arm of the missing pair. Kimber and Riley (1968) and Khush (1973) call this monoisosomy (symbol 20″+i′ 2n=41).
3. *Tertiary Monosomy.* As a result of pollen irradiation, two nonhomologous chromosomes are broken in the centromere region. Two arms of these non-homologues unite and form a **tertiary chromosome** with a functional centromere. The other two arms are lost. A plant fertilized with such pollen becomes a tertiary monosomic (Khush and Rick, 1966). Such a plant really is a **double monosomic** with a tertiary chromosome (TC) addition (symbol 19″+1′+1′+TC 2n=41) (Fig. 16.5).

Monosomics have been used extensively in wheat breeding for the purpose of **chromosome substitution.** The first **monosomic series** in wheat was established by Sears (1954) in the cultivar "Chinese Spring." The sources for such monosomics are:

1. *Asynapsis as caused by nullisomy.* This was the major source in wheat. Of 212 monosomics recovered, 114 (53.8%) were obtained from progeny of asynaptic nullisomic 3B (Sears, 1954).

[1]Disomic–Individuals (e.g., 6x) with complete sets of homologous chromosomes as opposed to monosomics (6x−1), nullisomics (6x−2), etc.

[2]Hemizygous–Genes not present as pairs of alleles but only once as a result of aneuploidy or loss of chromosome segments.

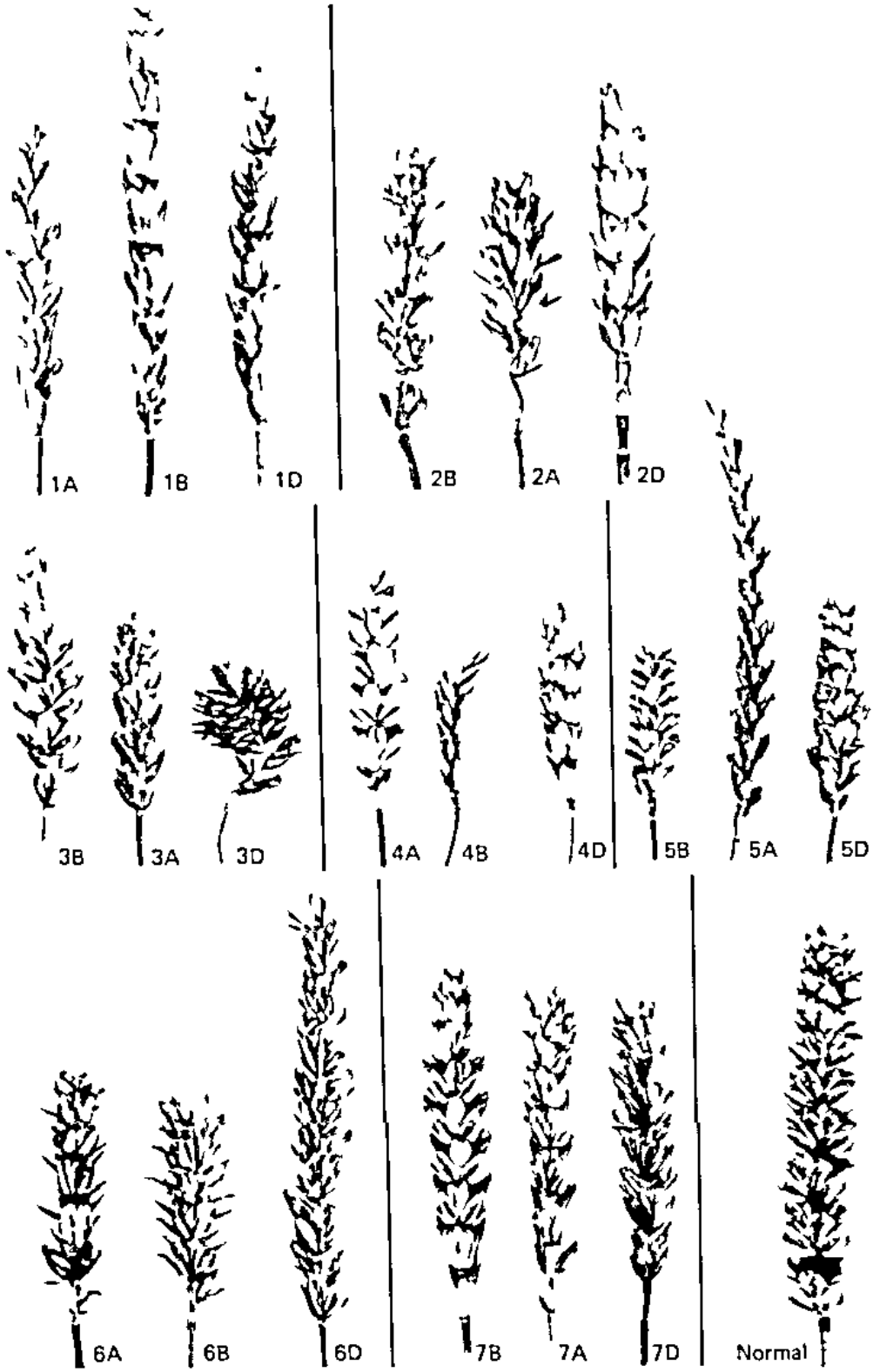


Fig 16 2 Seven homoeologous groups of 3 nullisomic Chinese Spring wheat sp kes each (A B D) compared with a normal spike (Courtesy of Dr Ernest R Sears USDA SEA Cereal Genetics Research Unit Columbia Missouri)

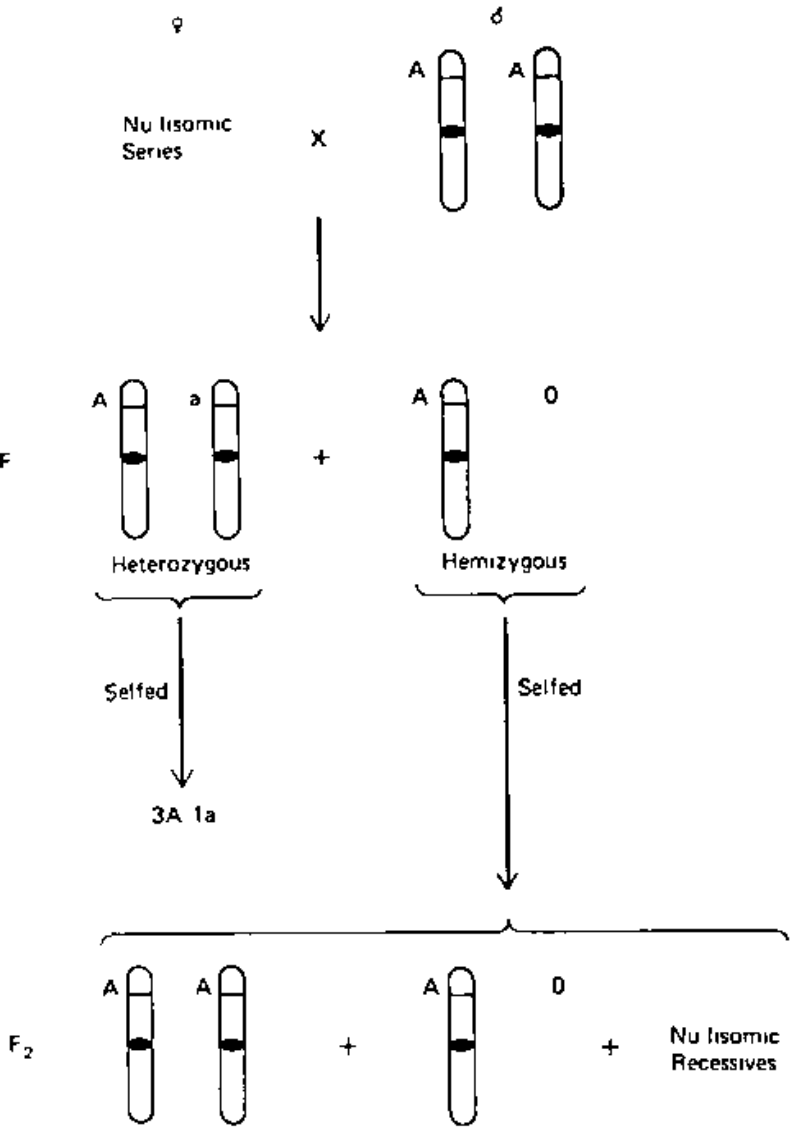


Fig 16 3 Schematic representation of nullisomic analysis Explanation in text

2 *Polyhaploid progeny* Of the 212 monosomics obtained by Sears (1954), 66 (31 1%) were derived from two different polyhaploid individuals
3 *Chromosome loss* as a result of **non-disjunction** during meiosis or during the early mitotic divisions of a diploid zygote
4 *Unequal chromosome distribution* (non coorientation) during meiosis of translocation heterozygotes (see Section 14 3)

Chromosome substitution lines in wheat have been produced since the first monosomic series became available Rueger et al (1976) defined chromosome substitution as the exchange of a single chromosome or a chromosome pair by chromosomes of the same complement (from a different variety, for instance) or by chromosomes of the complements of other species or genera (**alien chromosome substitution**). One of the purposes for chromosome substitution could be that, after demonstrating that disease resistance or some other desirable agronomic characteristic is conditioned by a gene or genes carried by a certain chromosome, this desirable chromosome could be substituted into an otherwise acceptable cultivar

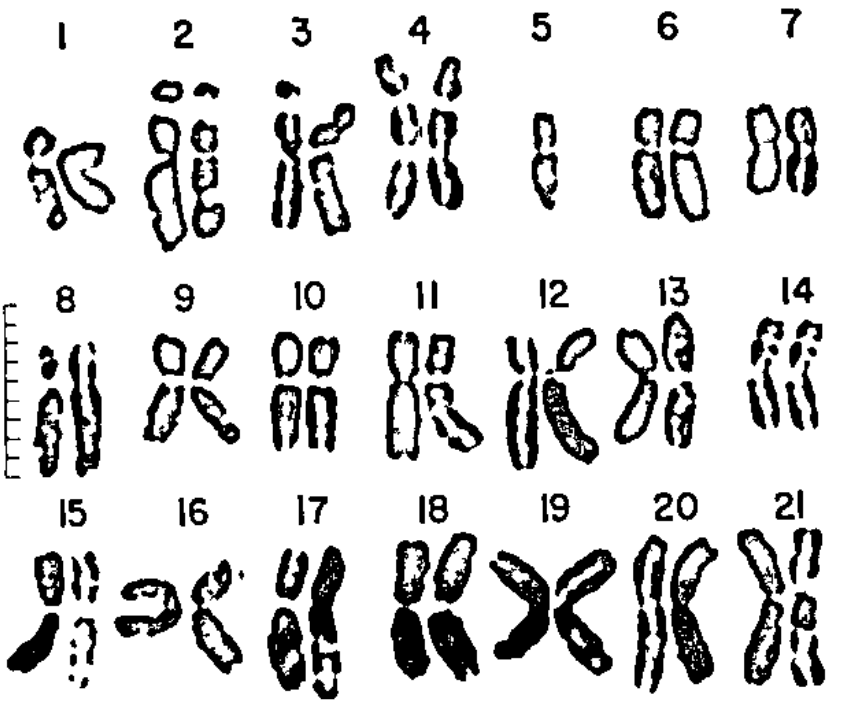


Fig 16 4 Karyotype of monosomic common wheat, *Triticum aestivum L* Chromosome pairs 1 to 4 are arranged according to the length of their satellites Chromosome 5 (4D) and pairs 6 to 21 are arranged according to the length of their short arms The photographs of the chromosomes were taken from a cell of a plant that was monosomic for chromosome 4D Scale units at left represent 1 μm (From Schulz Schaeffer and Haun 1961 Reprinted by permission of Verlag Paul Parey, Hamburg)

Assuming that "Chinese Spring" is that improved acceptable cultivar, it can be used as a monosomic female recipient for repeated backcrossing until the desirable chromosome of the male donor variety is transferred into the Chinese Spring back ground Such a technique takes advantage of the fact that monosomes have much greater transmission through male than through female gametes (Fig 16 6) In order to improve varieties in such a way, the monosomic series of Chinese Spring had first to be transferred to other such desirable cultivars This has been accomplished in more than a score of cultivars

In recent years chromosome substitution, with the help of aneuploids, has been used less because of the enormous effort in work and time required Usually, the same objective can be achieved through the backcross method (Hurd, 1976)

Monosomics were first found in tobacco (Clausen and Goodspeed, 1926) They also were detected in oats, tomato, maize, and cotton Since there is some homoeologous pairing between genomes in tobacco, trivalents occur in 25% of the monosomics In wheat, monosomics do not form trivalents

In humans the most common single abnormality in chromosomally abnormal fetuses is the Turner syndrome (45,X) (Hamerton, 1971b) It occurs in 0 03% of all female births This is the monosomy for the X chromosome Autosomal mono-

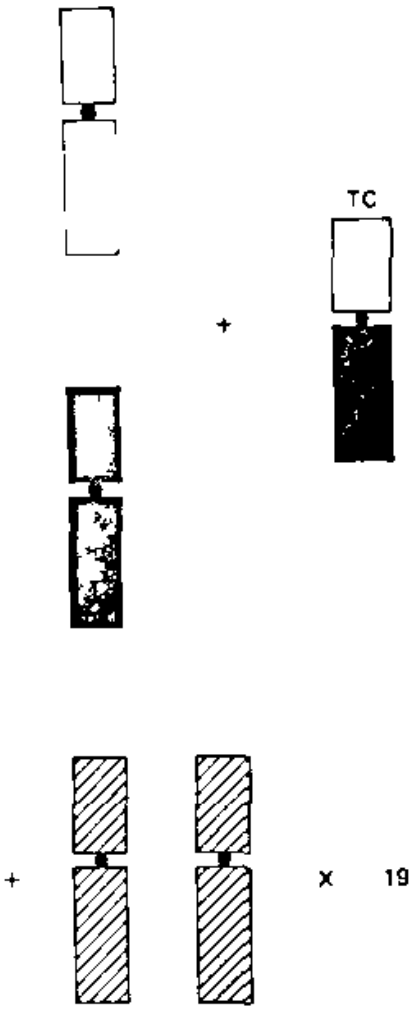


Fig 16 5 Schematic representation of tertiary monosomy The double monosomic condition is caused by the formation of a tertiary chromosome (TC)

somics in humans are very rare Al Aish et al (1967) reported one single complete G group monosomy but most others are mosaics

In *Drosophila* only one type of autosomal monosomic is known, the haplo-IV's or individuals with only one chromosome 4 This is a loss of 54 bands of a total of about 5000 in the entire *Drosophila melanogaster* complement Haplo-IV's are not as robust and healthy as normal flies There is no monosomy for chromosomes 2 and 3 Monosomics for the sex chromosomes so called XO flies, also exist in *Drosophila* They are males but are sterile XO types have also been reported in mice, and are female and fertile

As mentioned before monosomics in diploids are rare but have been isolated in maize as early as 1929 (McClintock, 1929c, Einset, 1943) Only more recent efforts have made it possible to use monosomics in genetic studies The establishment of the complete monosomic series in maize has been particularly helpful (Weber, 1974) Plewa and Weber (1975) used monosomic analysis for the study of fatty acid composition in embryo lipids of maize

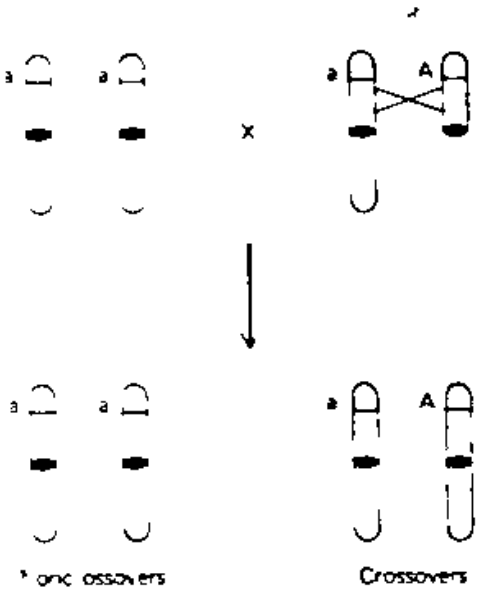


Fig 16 7 Monotelosomic facilitated mapping of genes in wheat

Most of the presently available telosomics of wheat are maintained as ditelosomics for which the chromosome concerned is represented by a homologous pair of telosomes (20″ + t″) (Sears 1966)

16 2 4 Trisomy

Trisomics are individuals with one or more (**doubletrisomics, etc**) extra chromosomes in an otherwise disomic chromosome complement Trisomy is very common in plants It was first discovered in Jimson weed by Blakeslee in 1921 (Chapter 1) Organisms of this kind are hyperploids There are five major kinds of trisomy recognized

1 *Primary Trisomy* The additional chromosome is completely homologous to one of the chromosome pairs of the complement
2 *Secondary Trisomy* The additional chromosome is a **secondary chromosome** or an isochromosome (see Section 10 3)
3 *Tertiary Trisomy* The additional chromosome is a translocated or **tertiary chromosome** consisting of two nonhomologous chromosome segments
4 *Compensating Trisomy* A chromosome is missing and is genetically compensated by two other modified chromosomes
5 *Telosomic Trisomy* The additional chromosome is a telocentric chromosome

These five types of trisomics can be distinguished cytologically in meiosis Given the proper zygotene pairing and conditions for chiasma formation, primary trisomics can form chains of three chromosomes in meiosis (Fig 16 8A) but never rings of three (Other possible trivalent configurations are shown in Fig 15 6A) Secondary trisomics can form rings of three (Fig 16 8B) and tertiary trisomics can form chains of five but never rings of five chromosomes (Fig 16 8C) Telosomic trisomics can be of several different constitutions If one deals with a monotelotrisomic (20″ + t2 ′ in wheat) a chain of three chromosomes can be formed but never a ring of three (Fig 16 8D)

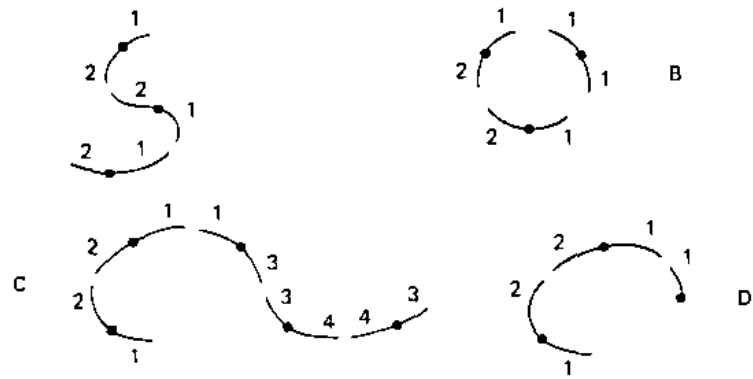


Fig 16 8A D Cytological identification of trisomics in meiosis (*A*) Primary trisomic (*B*) Secondary trisomic (*C*) Tertiary trisomic (*D*) Telosomic trisomic

16 2 4 1 Primary Trisomy The first detailed morphological description of a complete series of 12 primary trisomics (2n = 25) was presented by Blakeslee (1934) in *Datura* Each type in the series had its distinct fruit capsule morphology (Fig 16 9) Primary trisomics can also be distinguished morphologically in *Avena sativa* (Azael, 1973), *Avena strigosa* (Rajhathy, 1975) *Potentilla argentea* (Asker, 1976), *Pennisetum* (Manga, 1976), and in many other species Morpho logical differences between trisomics are not large enough to be distinguished in *Clarkia* (Vasek, 1956, 1963), *Collinsia* (Dhillon and Garber, 1960, Garber, 1964), *Triticum* (Sears, 1954), and *Nicotiana* (Clausen and Goodspeed, 1924) In maize only two trisomics, triplo-3 and triplo-5, could be identified morpholog-ically The rest could not be distinguished from each other nor from the disomics (McClintock, 1929a, McClintock and Hill, 1931, Rhoades and McClintock 1935) Additional series of primary trisomics have been established in maize (McClintock, 1929a), barley (Tsuchiya, 1958b, 1961), tomato (Lesley, 1932), rye (Kamanoi and Jenkins, 1962), and in other species The main source for primary trisomics is 3x types and subsequent hybridization between 3x and 2x types Other sources for obtaining primary trisomics are non-disjunction, progenies of triploids *and tetrasomics and translocation heterozygotes Primary trisomics have been* also obtained by the use of **ionizing radiation** and after colchicine and other chemical treatments Gottschalk and Mihutinovic (1973) in peas and Palmer (1976) in soybeans found certain desynaptic mutants that have a genetically con ditioned occurrence of meiotic univalents, which is a potential source of trisomics Primary trisomics are excellent tools for assigning linkage groups to specific chro-mosomes As a matter of fact, the most extensive genetic studies with the aid of aneuploids have been conducted with trisomics Geneticists made use of the fact that gene segregation in primary trisomes is different from that of any other chro-mosome in the complement

The crossing scheme for this kind of gene mapping is typically the following (Fig 16 10)

1 A plant homozygous recessive for a given gene (*a*) the linkage group of which is to be determined, is crossed to all plants of the primary trisomic series

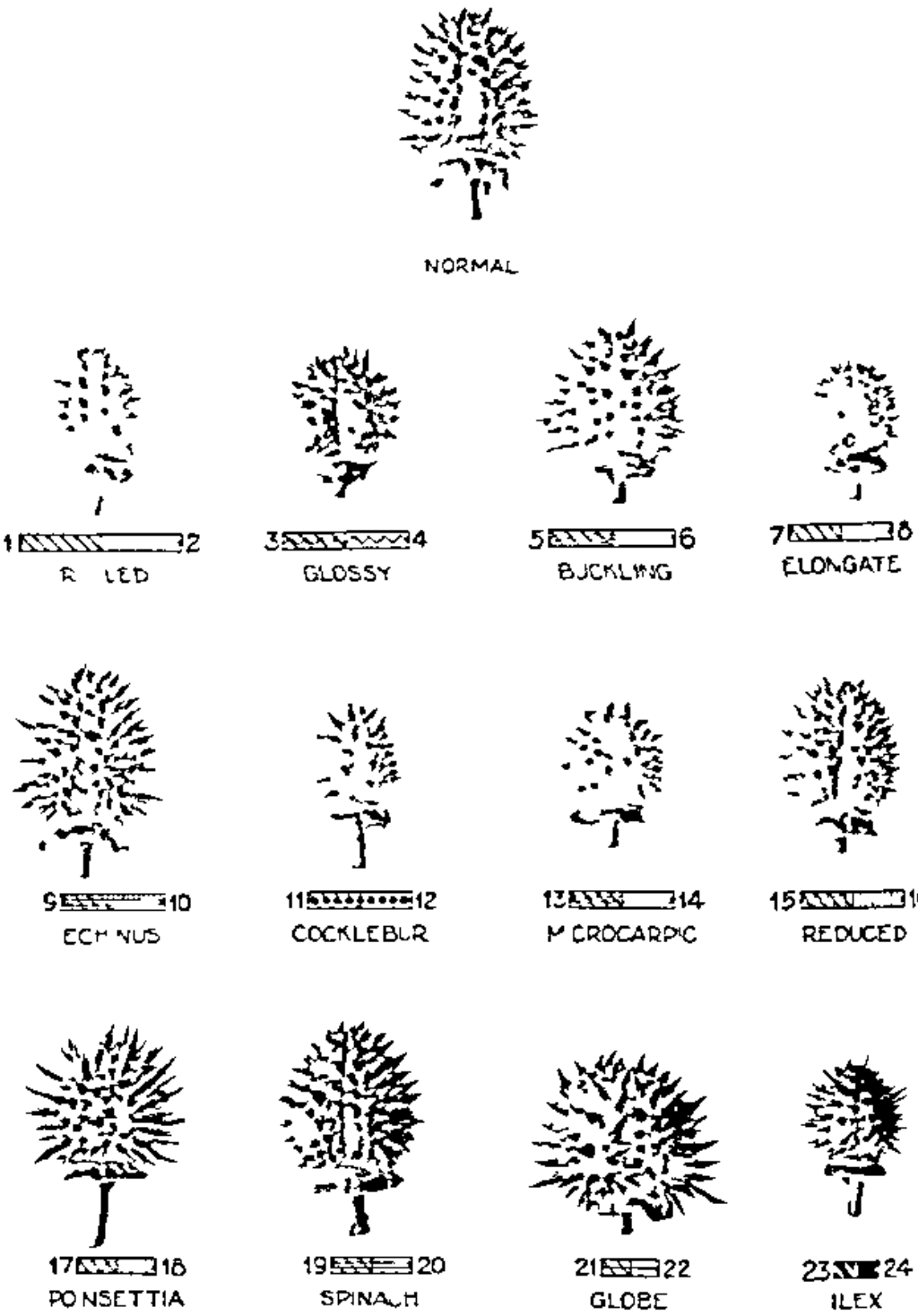


Fig 16 9 Fruit capsules and chromosomes (1 2 3 4 etc) of 12 primary trisomics of the Jimson weed *Datura stramonium* compared with a normal fruit capsule (From Blak eslee 1934 Reprinted by perm ss on of American Genetic Association Washington D C)

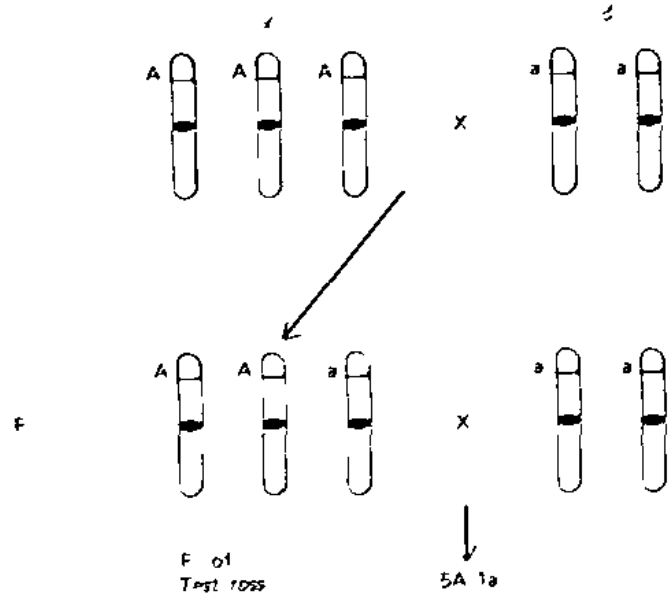


Fig 16 10 Gene mapping with the aid of trisomics

2 The trisomic F_1 plants are identified by cytological analysis or selected by morphological characteristics if that is possible They are then backcrossed to the homozygous recessive

3 The F_1 testcross plants are analyzed genetically for their segregation If there is a striking deviation from the normal phenotypic 1*A* 1*a* testcross ratio then the linkage group in question (marked by gene *a*) can be assigned to the primary trisome that produced this ratio

As seen in Fig 16 9, the trisomic carrying *AAA* produced *AAa* trisomics in the F_1 Genotypes of such trisomics are called duplex (Section 15 3 2 4) A plant with a duplex genotype produces the following gametes 1*AA* 1*a* 2*A* 2*Aa* The testcross ratio consequently results in 5*A* 1*a* This ratio may be modified depending on the distance of the *a* locus from the centromere permitting double reduction (Section 15 3 2 4) It also depends on the transmission of hyperploid gametes through the female In maize where this transmission was expected to be about 33% for chromosome 10, a testcross ratio of 3 8 1 was obtained for a trisomic of the $R_1R_1r_1$ genotype (R_1 57 Colored aleurone and plant) (McClintock and Hill, 1931)

This trisomic method for assigning linkage groups to chromosomes has been applied in *Datura* (Avery et al , 1959), *Antirrhinum* (Rudorf-Lauritzen, 1958), maize (McClintock and Hill, 1931), spinach (Janick et al , 1959), barley (Tsuchiya, 1958b, 1959a, 1959b, 1960, 1961, Tsuchiya and Takahashi, 1959, 1960), tomato (Rick et al , 1964), and *Petunia* (Smith et al , 1975)

The first discovered human trisomic syndrome was the one involving the G-group of chromosomes called mongolism or Down's Syndrome, which has a frequency of about 1 in 600 to 700 births It is also the most frequent autosomal aberration in humans This trisomy involves one of the smallest human chromosomes which is significant in that most larger duplications of gene complexes cannot very often survive The frequency of this syndrome at conception is estimated at 1 in 140 Spontaneous abortion is the explanation for the reduction in frequency of G tri-

somy at birth As a matter of fact, from 60% to 100% of all chromosomally abnormal fetuses are believed to be spontaneously aborted (Hamerton, 1971b) The incidence of mongolism is known to increase with the age of the mother The reason for this relationship is not yet entirely clarified, but one could conceive that the oocyte decreases in meiotic efficiency as maternal age increases Since the increase in G group chromosomes to five probably is caused by non-disjunction, one can speculate that the chromosomes with increasing age of the oocyte increase in stickiness which could prohibit their separation at anaphase and facilitate their combined transport to a single cell pole The smallness of the G chromosomes probably also contributes to their greater difficulty in separating Recent cytogenetic evidence seems to indicate that trisomy 21 can also originate from paternal chromosome non disjunction (Erickson, 1978) Many researchers have described *the clinical features of Down's Syndrome* (Øster, 1953, Penrose, 1961, Hanhart, 1960 Benda 1960 Beckman et al 1962, Gustavson, 1964, Penrose and Smith, 1966) Some of these features are severe mental retardation, saddle nose, and slanting eyes

Other examples of human primary trisomy are the Edward's Syndrome (E trisomy) Patau s Syndrome (D trisomy), and C trisomy or C-trisomy mosaicism The Edward s Syndrome (47,XX or XY, 18+) is the second most common autosomal trisomy found in live birth Based on three surveys of hospital new borns, the *overall minimum frequency is about 1 in 3 500* (Hecht et al , 1963, Marden et al , 1964 Taylor and Moores 1967) Eighty percent of the cases die within the first two months after birth and all usually die before one year of age The first case was reported in 1960 (Edwards et al) By 1971 about 150 individuals with this syndrome had been reported (Hamerton, 1971b) These cases show a high degree of mental retardation, short sternum (breastbone) and laterally flattened head The children are small and weak

The Patau Syndrome (47,XX or XY,13+) is characterized by deafness, myoclonic seizures (irregular involuntary contraction of muscles), eye defects, cleft palate (split roof of mouth) and mental deficiency Usually the largest of the D group chromosomes is involved in the duplication and consequently chromosome 13 is suspect (Fig 16 11) Autoradiograph and measurement studies have confirmed this observation (Giannelli 1965a and b Buchner et al , 1965 Giannelli and Howlett 1966 Yunis and Hook 1966) Patau first described the clinical features of this chromosome syndrome (Patau et al , 1960) About 75 children with this syndrome were described by 1971 The incidence is about 1 in 5000 live births According to Taylor (1968) the mean survival time is about 90 days There is no evidence that there is trisomy for any of the other D chromosomes

C trisomy and C trisomy mosaicism have been reported by several researchers (El Alfi et al 1963 Laurent et al 1971, Malpuech et al , 1972, De Grouchy et al , 1971) Presently this is the only trisomy of a large human chromosome that seems to be viable Most of the cases found up to now are mosaics Bijlsma et al (1972) and Kakati et al (1973) have attempted to establish this trisomy as a syndrome Since the wide application of chromosome banding has become a common practice, *C trisomy has been associated with individual C-group chromosomes* Trisomies 8

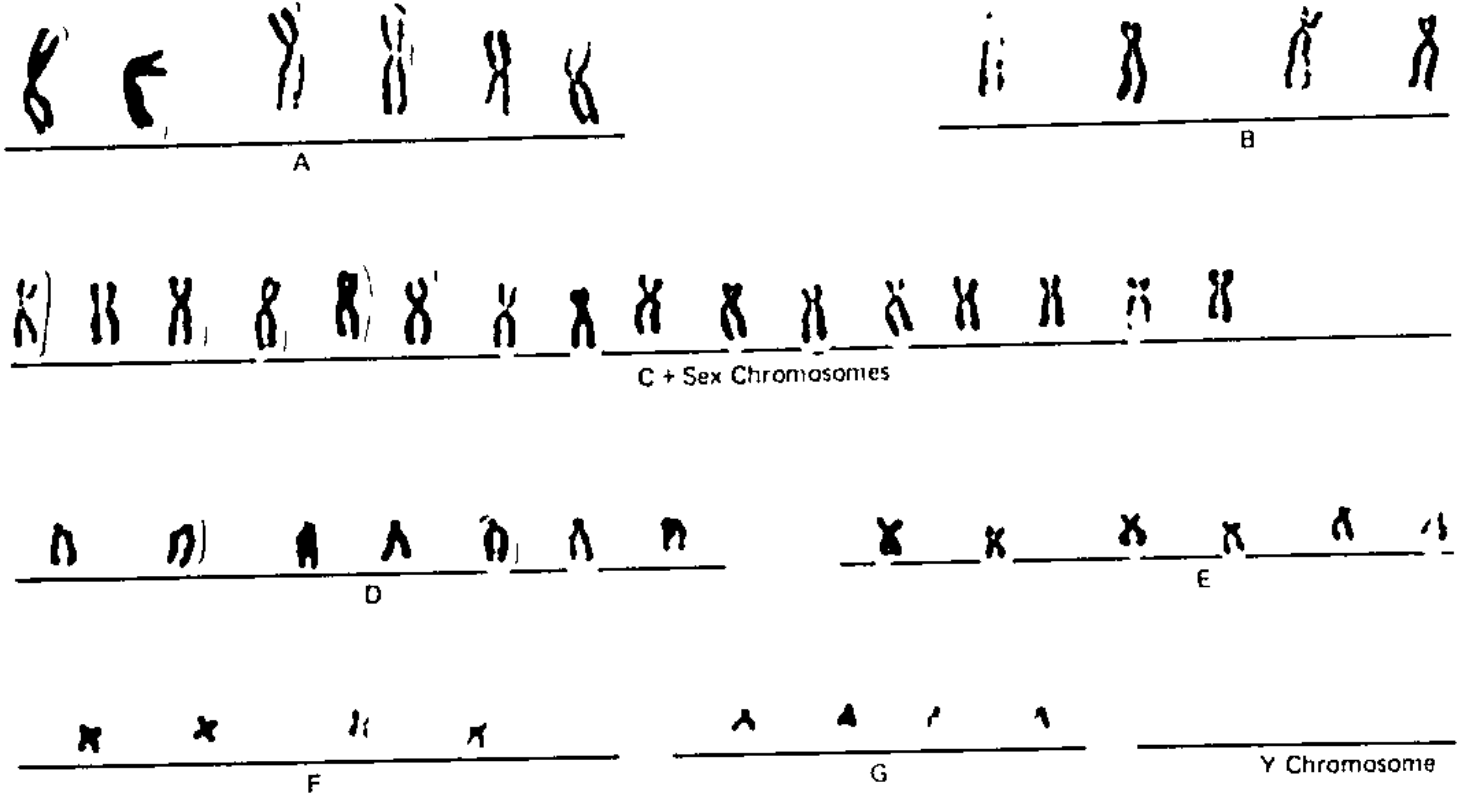


Fig 16 11 Karyotype of a primary D trisomic human with Patau syndrome (Courtesy of Dr Philip Pallister, Shodair Crippled Children's Hospital, Helena, Montana)

and 9 are well established Only a few cases of complete trisomy 8 have been described (Caspersson et al , 1972 Kakati et al , 1973, De Grouchy et al , 1974, Jacobsen et al 1974, Sperber, 1975, Gagliardi et al 1978) A wide variety of congenital malformations was reported in these individuals Mace et al (1978) summarized the present situation for trisomy 9 About 25 cases of trisomy for the short arm of chromosome 9, two cases of complete trisomy 9, and one case of a mosaic condition have been reported

Sex-chromosome trisomics have a relatively high frequency in man One of the earliest clinical syndromes linked to chromosome aneuploidy after the establishment of the right chromosome number in humans was *Klinefelter s Syndrome* At least two X chromosomes and one Y (XXY) are a common feature of all these syndromes But other combinations such as XXXY, XXXXY, XXYY, and XXXXYY have also been observed Mixoploids such as XXY/XX, XXY/XY, XXY/XXXY, and XXXY/XXXXY also exist The incidence of live births in the population is about 1 in 500 This is a higher frequency than mongolism XXY males show incomplete sexual expression Some Klinefelter males have been reported to have mild mental or psychotic disorders (Mosier et al , 1960; Anders et al , 1968)

Trisomics for the X chromosome (47,XXX) are females with double sex chromatin Seventy cases had been observed by 1971 They do not have any sexual abnormalities According to Lubs and Ruddle (1970), they occur one in 727 female new borns The **double Y syndrome** (47,XYY) has often been associated with supermaleness Non-disjunction of Y chromosomes in anaphase of meiosis II is the most likely explanation for its origin According to Lubs and Ruddle (1970), the incidence of this trisomy in male infants was one in 570 They pooled the results of three studies surveying 6,746 male infants This trisomy is similar to the 48,XXYY tetrasomy that was found in high proportion among males in institutions for the criminally insane (Casey et al , 1966, 1968) Several workers found evidence that XYY trisomy was often also associated with aggressive, tall males who were in prison (Close et al , 1968, Telfer et al , 1968) However, Witkin et al (1976) maintained that according to their studies there is no evidence that XYY men are especially aggressive Hamerton (1971b) suggested that males with the double Y syndrome suffer from considerable inherent psychosocial disorders that make it difficult if not impossible for them to adjust to a normal social environment Sex chromosome syndromes are generally less upsetting to the genome balance than autosomal ones because Y chromosomes are almost entirely heterochromatic and genetically inert (Section 5 2) and the X chromosomes are, with the exception of one, all heterochromatinized (Section 5 3 1)

In animals a number of trisomic cases have been reported Trisomy of a small acrocentric autosome in chimpanzee resembled Down's Syndrome (McClure et al , 1969) and XXY sheep showed testicular hypoplasia (arrested development of testes) typical for Klinefelter's Syndrome (Kilgour and Bruere, 1970) Mouse trisomics were found to be phenotypically normal but sterile or semisterile (Cattanach 1964, Griffen and Bunker, 1964, 1967) In *Drosophila melanogaster*, chromosome 4 trisomy (or triplo-IV) is viable and fertile in the female sex (Sturtevant, 1936) This is the only chromosome in *Drosophila* that can survive in the triplicate

state It constitutes only about 2% of the total chromosome complement Grasshoppers were found to be trisomic for various autosomes (Callan 1941 Lewis and John 1959 Hewitt and John 1965 Sharma et al 1967)

16 2 4 2 Secondary Trisomy Secondary trisomy sometimes occurs in the progeny of normal plants but mostly among offspring of plants with univalent chromosomes (Burnham, 1962) Misdivision of the centromere is the origin of the isochromosome which distinguishes secondary trisomics (Section 10 3)
In *Datura* (2n = 24) 24 different secondary trisomics are possible If each chromosome arm is numbered the types are

1 2 1 2 1 1 or 1 2 1 2 2 2
3 4 3 4 3 3 or 3 4 3 4 4 4
5 6 5 6 5 5 or 5 6 5 6 6 6
etc

Fourteen had been identified by Blakeslee and Avery by 1938 Secondary trisomics are the least investigated among the four major kinds mentioned above (Section 16 2 4) Khush (1973) stated that the production of isochromosomes is predominantly a chance event, although experimental methods can be used to produce them Sen (1952) obtained two monoisodisomics in tomato in progenies of pollen treated with formaldehyde and ammonia vapor Khush and Rick (1967a) received five monoisodisomics from pollen irradiation Such plants can serve as a source of secondary trisomics Other secondary trisomics are known for maize (Rhoades 1933) tomato (Khush and Rick 1968b 1969) wheat (Sears 1954) and oats (Rajhathy and Fedak, 1970 Rajhathy 1975)
Advanced studies with secondary trisomics have been carried out by Khush and Rick in tomato (2n = 24) They isolated 9 of the possible 24 secondary trisomics which brings the total number for tomato up to 10 (Moens, 1965) They found that most of the morphological characteristics of the primary trisomics are exaggerated in the secondaries since one arm is represented four times in the chromosome complement rather than only three times as in the primaries The segregation ratios of the secondary trisomics are different from those of the primary trisomics In the primary trisomics the three homologous chromosomes can entirely substitute for each other accounting for the unique trisomic segregation ratios But in secondary trisomics the segregation is primarily disomic but complicated by the existence of an extra isochromosome The transmission of the isochromosome varies depending on the chromosome arm involved. In *Datura* that transmission to the progeny after selfing ranged from 2% for the 1 1 secondary to 31% for the 5 5 secondary (Blakeslee and Avery 1938) Any spore that receives an isochromosome instead of a normal chromosome aborts because it is deficient of one chromosome arm The segregation testcross ratio of secondary trisomics of *AAAa* constitution deperds on the location of the recessive marker If the recessive marker is located on the isochromosome, none of the trisomic progeny will be

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Monoisod somic–one chromosome is missing but is replaced by an isochromosome for one of the arms of its homologue *Tomato* has 2n = 24 The monoisodisomic symbol is 23″+i1″

recessive If the recessive marker is located on one of the normal homologues, the expected trisomic testcross ratio will be 1 1 In Khush and Rick's (1968b, 1969) data, the percentage of recessive secondary trisomics in the testcross F_1 was lower than expected because of lower viability of these trisomics

Khush and Rick could clarify the relationship between four tomato chromosomes and their corresponding genetic linkage maps by the trisomic method using secondary trisomics in tomato

According to Khush (1973), secondary trisomics can be used as efficient tools in linkage mapping The segregating progenies can give data on the chromosomal and arm location of a genetic marker, the centromere position, and the proximity of the marker to the centromere

Feldman (1966) obtained six doses of the pairing suppressor *Ph* of wheat by producing trisosomic 5BL ($20''+1'''$) and was, thereby, able to deduce the method of action of this important gene

16 2 4 3 Tertiary Trisomy As mentioned, the additional chromosome in a tertiary trisomic is a tertiary or translocation chromosome (Section 14 3) They regularly occur in the progeny of translocation heterozygotes In spite of their cytogenetic value, they have been studied in only a few species Avery et al (1959) established 30 different tertiary trisomics in *Datura* Other tertiary trisomics were identified in *Oenothera* (Catcheside, 1954), barley (Ramage, 1960, Prasad and Das, 1975, Prasad, 1976), maize (Burnham, 1930), rye (Sybenga, 1966), tomato (Khush and Rick, 1976b), and in peas (Müller, 1975) As mentioned in Section 16 2 4 1, primary trisomics are ideal tools for assigning genetic markers and entire linkage groups to specific chromosomes Tertiary trisomics and telotrisomics are tools to determine arm location and approximate distance from the centromere In a tertiary trisomic, the genetic ratios are modified only for genes located in one chromosome arm, since it has only one extra arm or part of such an arm for a particular chromosome

In a tertiary trisomic test, the recessive gene to be located, (*e g , a*) has been previously identified with a specific chromosome by the primary trisomic test (Fig 16 10) The recessive gene is then incorporated into the corresponding tertiary trisomic, which is duplicated for one arm or part of one arm of the identified chromosome One of the two normal homologues of the resulting trisomic will carry the recessive gene (*a*) while the other homologue and the tertiary chromosome will carry the normal alleles (Fig 16 12) If crossing over is ignored, the disomic fraction in the testcross progeny of such a trisomic may segregate 1*A* 1*a* and all the trisomics would be normal (*A*) Such ratios would indicate that the gene under investigation is located in the duplicated arm If the gene is not located in the duplicated arm, both the disomic and the trisomic fraction of the progeny will segregate 1*A* 1*a*

In the tomato, seven tertiary trisomics were studied, five of which were used for genetic tests Marker genes were assigned to specific arms of chromosomes 1, 4, 5, 7, 9, and 10

Ramage (1964, 1965) described a method by which one could use tertiary trisomics with genetic recessive male sterile genes (*ms*) in the production of hybrid

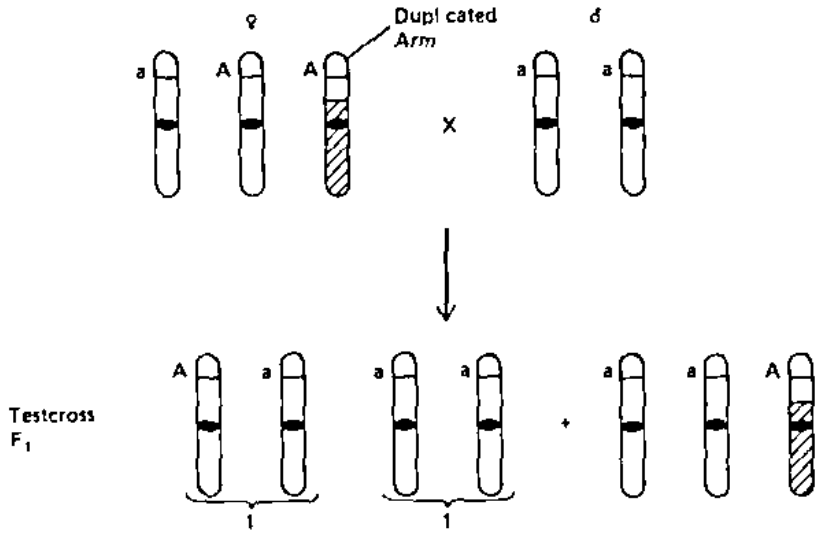


Fig 16 12 The tertiary trisomic test

barley He called this method the **balanced tertiary trisomic system** According to Ramage, balanced tertiary trisomics are "tertiary trisomics set up in such a way that the dominant allele of a marker gene, closely linked with the interchange breakpoint, is carried on the extra chromosome, and the recessive allele is carried on the two normal chromosomes that constitute the diploid complement" If the dominant marker allele is responsible for male fertility (*Ms*), the tertiary trisomic has the genetic constitution *Ms ms ms* (Fig 16 13) All functional pollen with a normal haploid chromosome complement from such a plant carries only the recessive *ms* gene, since *Ms ms* pollen is not able to compete Pollen with only the translocated chromosome (*Ms*) carries a duplication and a deficiency (Dp-Df) and is not viable Since there is also lowered transmission of the extra translocated *Ms* marked chromosome through the egg, the progeny consists of only 30% balanced tertiary trisomics but 70% disomics In Fig 16 13, the extra translocated chromosome has a second marker, in this case a dominant gene for a red plant color All balanced tertiary trisomics have a red plant phenotype and are male fertile All diploids would have a green plant phenotype and would be male sterile The red marker gene or any other similar marker can be used for separating the male sterile from the male fertile plants

However, all of the presently available commercial hybrid barleys do not have to rely on any extra color marker gene (Ramage, 1975) The male parent rows are a normal barley cultivar, which is a good pollen producer The female parent rows are the selfed progeny of trisomic plants (Fig 16 13) containing about 30% male fertile balanced tertiary trisomics (*Ms ms ms*) and 70% male sterile diploids The male fertile trisomics are shorter, weaker, and later flowering Therefore, this female parent produces almost pure stands (95% to 100%) of male sterile diploid plants in commercial hybrid seed production In order to completely assure crowding of the male fertile trisomic individuals in the female parent rows, the seed from the balanced tertiary plants is sown at a specially heavy rate (25 to 30 kg/hectare)

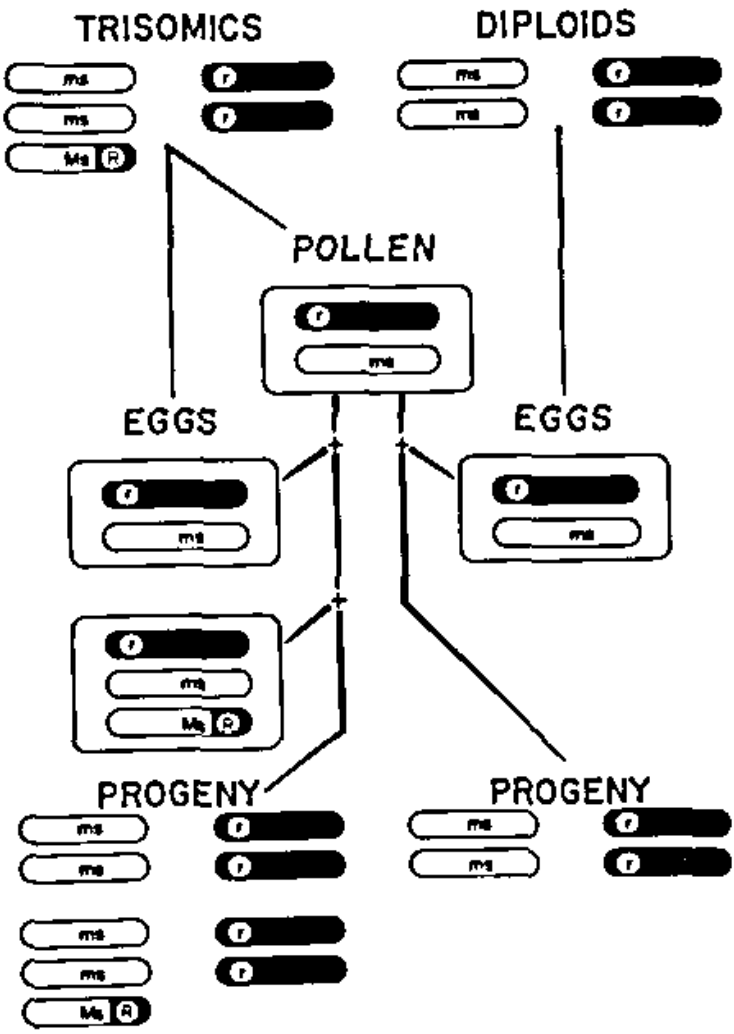


Fig 16 13 Breeding behavior of a balanced tertiary trisomic marked with a dominant mature plant character (*Ms*) (From Ramage 1965 Reprinted by permission of the American Society of Agronomy Madison Wisconsin)

16 2 4 4 Compensating Trisomy A compensating trisomic was found by Blakeslee (1927) in the progeny of a translocation heterozygote of *Datura* that involved two reciprocal translocations and a ring of 6 chromosomes The translocation involved the chromosomes 1 2 5 6 and 9 10 of *Datura* (2n=24) The translocated chromosomes were of the constitution 10 2 1 9 1 6, and 5 2 (Fig 16 14) If chromosomes 9 10 1 9 5 6 and 2 5 of the translocation ring combine with a normal gamete a plant results with only one 1 2 chromosome the missing 1 2 chromosome being compensated for by the 1 9 and 2 5 chromosomes Such a

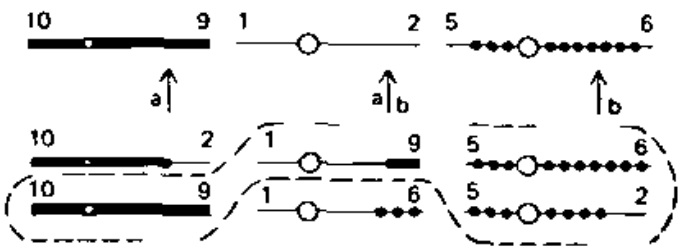


Fig 16 14 Illustration of the possible origin of compensating trisomy (From Burnham 1962)

plant is trisomic for the 5 and 9 chromosome segments A chain involving 7 chromosomes may occur in the first meiotic division of such a plant 9 10-10 9 9 1 1.2 2.5-> 6-6 5

16 2 4.5 Telosomic Trisomy In telosomic trisomy the additional chromosome (6x+t, 2x+t, etc) is a *telosome* which is homologous to one chromosome arm in the otherwise normal disomic complement In wheat (2n=42) nomenclature this is called a monotelotrisomic or is symbolized as 20"+t2 If the telosome is identified the chromosome number can follow the symbol For instance if the extra telocentric wheat chromosome is 5A the symbol would be 20"+t2 5A Telotrisomics have been reported in maize (Rhoades 1936 1940) *Datura* (Blakeslee and Avery 1938) tobacco (Goodspeed and Avery 1939) wheat (Moseman and Smith 1954) barley (Tsuchiya 1960 Singh and Tsuchiya 1975) rye (Kamano and Jenkins 1962) and tomato (Khush and Rick 1968c) In maize and wheat the telotrisomics could be used to determine the arm location of various genes In most species it is difficult to identify the telosomes as to their arm homology However in tomato and maize identification is possible because accurate pachytene analysis can be carried out (Section 2 2) Genetic segregation ratios for telotrisomics are very similar to those in tertiary trisomics They are only modified for the markers located on the duplicated telocentric arm
By 1968 six monotelotrisomics were discovered and identified in tomato Inheritance studies with three of them facilitated arm assignment of marker genes In barley 7 monotelotrisomics were used to analyze over 50 genes on 7 chromosomes Since telocentric chromosomes are shorter than complete chromosomes the transmission rate was higher through female gametes than in primary trisomics

16 2.5 Tetrasomy

Tetrasomics are organisms in which one chromosome is present four times in an otherwise disomic chromosome complement (e g 6x+2) In wheat the symbol is 20"+4 Sears (1952c) used tetrasomic wheats in order to establish the so-called homoeologous groups in that species Particular tetrasomics after combination with nullisomics can cancel the morphological expression of certain nullisomics From the study of nullisomic tetrasomics, Sears concluded that there are seven such chromosome groups of three homoeologous chromosomes each Each tetrasomic compensated to some degree for either of the other two nullisomics Sears synthesized all 42 possible nullisomic tetrasomic combinations within each of the 7 homoeologous groups, and each showed some superiority over the nullisomics In many cases the compensation was complete After this study the chromosomes of wheat were reclassified from a Roman numeral numbering system (I–XXI) to an Arabic numbering system with capital letters following numbers to designate genomic relationships (1A 7A 1B-7B 1D-7D) Similar chromosome compensation between nullisomics and tetrasomics has also been demonstrated in oats

Part VIII
Variation in Chromosome Function and Movement

In Parts V, VI, and VII the possible deviations from the normal chromosome types, structure, and number were described and discussed In the forthcoming three chapters, the variation in the function and movement of the chromosomes is considered

Chapter 17
Variation in Function of Autosomes

Both chromosome function and movement are highly coordinated and precisely efficient processes In this context Swanson (1957) commented "The fact that cell division is not a unitary process means that the steps that normally occur in orderly succession are subject to disturbance and open to attack The natural causes of upsets in cell and chromosome behavior can be examined, as well as their consequences to the particular individual and to the population at large "

17 1 Somatic Segregation

Each cell division normally leads to the formation of two cytologically and genet ically identical daughter cells But, due to cytological and genetical disturbances, cell division can lead to unlike daughter cells and, consequently, to unlike tissues The results are phenomena like mosaicism, chimeras, variegation, and mixoploidy Genetic mosaics caused by intrachromosomal changes, for instance, are the result of **somatic crossing over.**

17 1 1 Somatic Crossing Over

Somatic crossing over occurs during mitosis of somatic cells and leads to the segregation of heterozygous alleles Its prerequisite is somatic chromosome pairing (i e , pairing of homologous chromosomes) as discussed in Section 9 2 Somatic crossing over, like meiotic crossing over, occurs in the four strand stage of chromosomes and is common in many dipterans If, for instance, a certain tissue of the fly *Drosophila* has a gene for yellow body color represented in a heterozygous condition (*Bb*) and crossing over occurs between the centromere and this gene, then a daughter cell can originate that carries the gene *b* in a homozygous recessive condition (Fig 17 1a) The tissue that develops from this cell would show a yellow (*bb*) **single spot.** In a more critical test, two recessive genes for body color, *a* and *b*, are located on the same chromosome and are involved in somatic crossing over The crossover location is between the centromere and the first gene (*a*) The result can be two adjacent cells of which one is homozygous for gene *a* (*aa*) and the other for gene *b* (*bb*) The tissues that develop from these two adjacent cells will form a so-called **twin spot, twin patch,** or **double spot** (Fig 17 1B) Figure 17 2

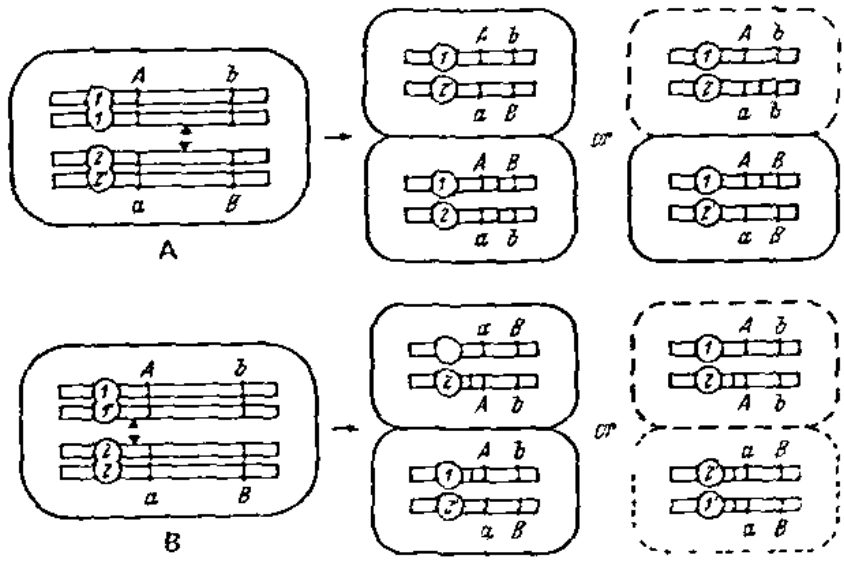


Fig 17 1A and B Diagram explaining somatic crossing over and its genetic consequences in a double heterozygote (*Ab/aB*) (*A*) Crossing over is located between the two loci *A* and *B* which results in a *bb*-spot (*B*) Crossing over is located between the centromere and the first locus, *A*, which results in a twin spot, *aa bb* (From Rieger et al 1976)

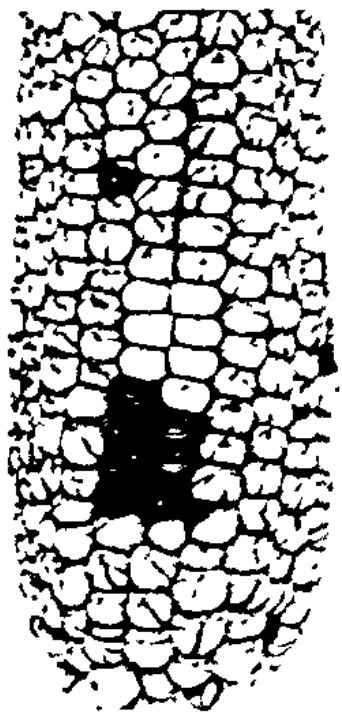


Fig 17 2 Twin spot in maize, consisting of light variegated and self colored kernels, on a variegated ear (From Brink and Nilan, 1952 Reprinted by permission of the Genetics Society of America, Austin, Texas)

shows a twin spot in the ear of maize Somatic crossing over has been demonstrated in *Drosophila* (Stern, 1936) maize (Jones, 1937), and asexual fungi (Pontecorvo, 1958) (Haendle, 1971a 1971b, 1974) showed that somatic crossing over in *Drosophila* can be induced by x rays and that it is dependent on the dosage Vig (1973a 1973b) studied the effect of inhibitors of DNA synthesis on the induction of somatic crossing over in soybeans Somatic crossing over was induced only by those chemicals (caffeine and actinomycine D) that are known to allow rejoining of chromosomes *He saw this as evidence that somatic crossing over is caused* by a specific event in DNA repair rather than by mere inhibition of DNA synthesis Soybean seems to be an ideal object for the study of somatic crossing over Its frequency of somatic crossing over is almost 10 times higher than in tobacco (Evans and Paddock, 1976) Twin spots composed of a dark green (Y_{11} Y_{11}) and a yellow (y_{11} y_{11}) component can be observed adjacent to each other on the light green (Y_{11} y_{11}) leaves in the areas of complementary exchange for these genes Vig suggested that this genetic system in soybeans should be given a wider try at least for preliminary testing of the effect of mutagens

Zimmerman et al (1967) suggested that somatic crossing over may be responsible for some form of cancer in humans Somatic crossing over leads to homozygosis of recessive genes that when phenotypically expressed may be detrimental or lethal and might lead to malignant growth

17 1 2 Chromosomal Chimeras

These are cytogenetically heterogeneous tissues that lie side by side in an organism and lead to the formation of mosaics They are caused by changes in chromosome structure or number and can therefore be called **chromosomal chimeras.** A chimera can be defined as "an organism usually a plant, that is not genetically uniform throughout" (Cramer, 1954) In chromosomal chimeras, distinct adjacent tissue layers have different chromosome structures or numbers They have been reported in *Nicotiana Solanum Datura* and *Crepis* etc They may be classified according to their different structural origin

1 sectorial chimeras (Fig 17 3A)
2 mericlinal chimeras (Fig 17 3C)
3 periclinal chimeras (Fig 17 3B)

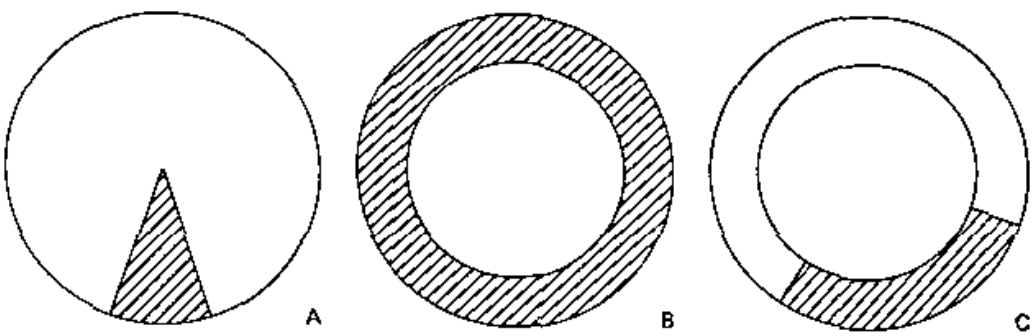


Fig 17 3A C Schematic illustration of chromosomal chimeras (*A*) Sectorial chimeras (*B*) Periclinal chimeras (*C*) Mericlinal chimeras (From Swanson 1957 Redrawn by permission of Prentice Hall Inc Englewood Cliffs N J)

In sectorial chimeras, different tissues occupy distinct sectors of the plant and are not limited to tissue layers Instead, the heteroploid tissue extends from the center of the affected plant part (root, stem, or leaf) to the epidermis This type was discovered and described in *Datura* (Blakeslee et al 1939 1940) In this type one branch of the plant may become tetraploid and another diploid depending on the origin of specific branches In an investigation by *Brumfield (1943) on the faba bean (Vicia faba) and Crepis involving chromosome rearrangements induced by x* rays, most of the chimeras obtained were of the sectorial type The prevalence of sectorial chimeras and the almost complete absence of periclinal chimeras in this study seemed to be caused by the method of treatment that involved x-rays Only single apical cells were affected by the treatment that supposedly gave rise to a chimeral sector behind the apical meristem The sector usually involved about one-third of the root s cross section including root cap epidermis cortex, and central cylinder *Figure 17 4, for instance* shows how *from a stem with a 2x-4x* sectorial chimera (A), pure 4x (B), sectorial (C), pure 2x (D), periclinal (E), and mericlinal (F) branches can arise If the plant can be propagated asexually, one could produce plants that are composed entirely of tissues with different chromosome numbers Much of the information about the behavior of sectorial chimeras stems from **gene differential chimeras** (Rieger et al , 1976) These are chimeras that could arise, *for instance, from somatic mutation of a gene to its recessive allele* A periclinal chimera can arise from a sectorial one, such as if a superficial strip of the 2x component overlaps the 4x component (Fig 17 4) If such an overlap is extensive and the budding branch originates within its periphery, such a newly originated branch will be periclinal (Neilson-Jones, 1969)

Mericlinal chimeras (Fig 17 3C) are interrupted periclinal chimeras in which, for instance, only part of the covering layer or epidermis is involved in the tissue differentiation Swanson (1957) believed that this type is probably the most commonly found although the most unstable insofar as perpetuation is concerned

Periclinal chimeras (Fig 17 3B) are probably the most stable ones They may have

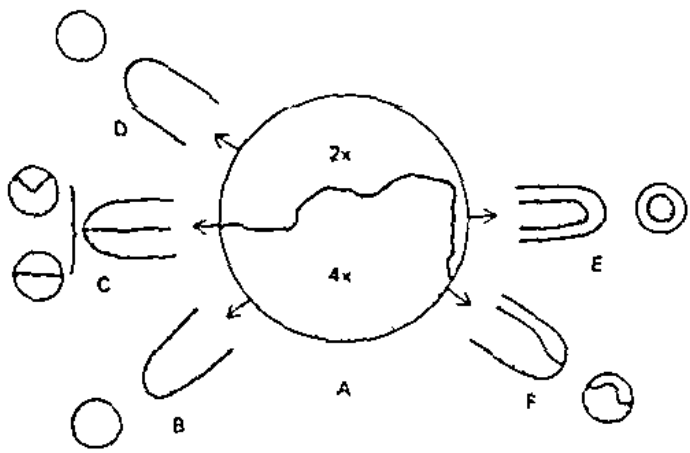


Fig 17 4 Possible origin of uniform branches (B and D), sectorial (C), periclinal (E), and mericlinal branches (F) from the stem of a sectorial chimera (From Neilson-Jones, 1969 Reprinted by permission of Methuen and Co , LTD , London)

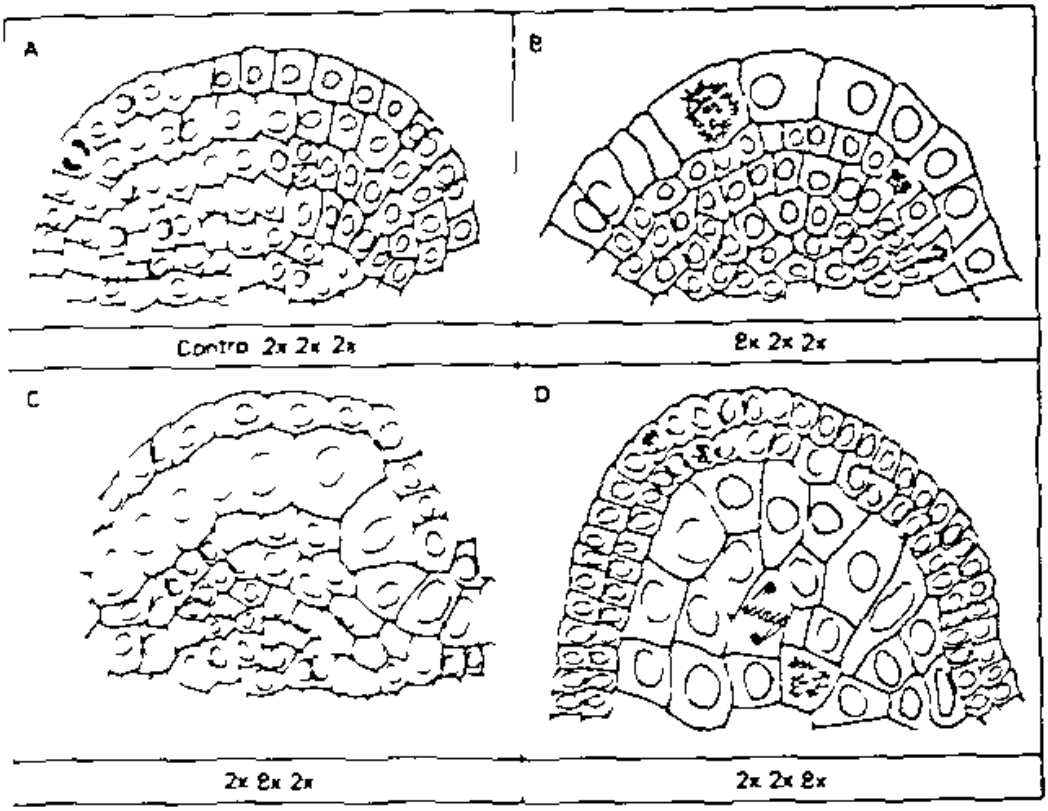


Fig 17 5A–D Drawings of longitudinal sections through the shoot apex of diploid Jimson weed *Datura stramonium* L., showing three layers of periclinal chromosome chimeras (A) Diploid layers (2x) of first tunica, second tunica, and corpus (B) Octoploid first tunica (8x) diploid second tunica (2x) and diploid corpus (2x) (C) Diploid first tunica (2x) octoploid second tunica (8x) and diploid corpus (2x) (D) Diploid first and second tunica (2x) and octoploid corpus (8x) (After Satina et al., 1940 Redrawn by permission from Colchicine in Agriculture, Medicine, Biology, and Chemistry by O J Eigsti and P Dustin, Jr © 1955 by the Iowa State University Press, Ames, Iowa 50010)

entire chimeral layers of tissue that can be one, two, or more cells in depth The differing tissue can occupy either the core of the plant structure, it can be sandwiched between two layers, or it may involve the covering layer such as the epidermis Most chimeras produced by colchicine are of the periclinal type Such chimeras were described in *Datura* (Satina et al., 1940; Satina and Blakeslee, 1941) The cells that are affected by the spindle fiber poison, colchicine, are in the actively dividing meristem. The stage most susceptible to the action of colchicine is late mitotic metaphase The cells of one particular germ layer are all in metaphase, while the neighboring layers are in earlier or later division stages Consequently, only one cell layer will be affected by the colchicine, while the others will remain unchanged (Neilson-Jones 1969) In *Datura* periclinal chimeras of 2x+4x and 2x+8x constitution were produced by treating germinating seeds with colchicine The diagrams of longitudinal sections through the shoot apex of *Datura* in Fig 17 5 show the various combinations of different ploidy levels in the three germinal layers of the shoot apex (first tunica, second tunica, corpus, the tunica is the outermost of the growth regions of the apical meristem) Similar chimeras were studied by Dermen (1941, 1945, 1953, 1960) in peach, apple, and

cranberry Blakeslee (1941) explained the use of polyploidy in periclinal chimeras to label the different germ layers as to their contribution to the development of a given plant organ Chromosomal chimeras also have been observed as part of normal tissues and are often referred to as polysomaty (Section 17 1 4)

17 1 3 Chromosomal Mosaics

In men and animals the term **mosaic** is generally used for a phenomenon that is similar to a chimera in plants **Chromosome mosaics**, like chromosomal chimeras may have cells differing *in chromosome structure or number* Many *human chromosomal mosaics* have been mentioned in previous chapters They are often referred to as mixoploids An example of aneuploidy as a normal phenomenon in a human tissue is the *endometrium* (mucous membrane lining the uterus) in which chromosome numbers range from $2n=17$ to $2n=103$ (Hughes and Csermely 1966) Tetraploid cells have been found along with diploid ones in rat liver (*Alfert and Geschwindt* 1958) *and in certain mammalian brain cells, including* the Purkinje cells of the cerebellum (Cohn 1969)

17 1 4 Polysomaty

This phenomenon is actually identical to endopolyploidy which was discussed earlier (Section 9 1) **Polysomaty** is a *term* that was coined by Langlet (1927) to designate normal tissues that contain diploid and polyploid cells adjacent to each other The terms "chimeras" and "mosaics" are generally used for anomalies, but the literature is not consistent The terms "chromosome chimeras" and "chromosome mosaics" do not necessarily always imply changes in chromosome number *only, but also in chromosome structure The term "polysomaty," however,* is restricted to tissues in which euploid chromosome numbers at various ploidy levels occur together Such change in ploidy can be explained by the origin of polysomaty through the process of endomitosis (Section 9 1, Fig 9 1) Endomitosis always implies that polyploidization occurs in differentiating tissue This is another form *of somatic segregation that occurs both in animal and plant tissues Polysomaty* apparently was first discovered in plants by Stomps in 1910 He observed that many cells in the periblem (cortical region) of the spinach root regularly had twice the typical somatic number This finding was confirmed by Litardière (1925) in hemp More extensive studies demonstrating polysomaty in spinach ($2n=12$) have been carried out by Lorz (1937), Gentcheff and Gustafsson (1939), and Berger (1941), who showed that the degree of polysomaty extended from 4x to 8x to even 16x *in* some cases Other plant species in which polysomaty has been demonstrated are maple (Meurman, 1933), melon (Ervin, 1941), and 39 species and varieties of Liliales (Sen, 1973)

Polysomaty in animals was first discovered by Holt (1917) in the alimentary tracts of the mosquito, *Culex pipiens* Hertwig (1935) *showed that the phenomenon* occured in the nurse cells of the *Drosophila* ovary where nuclear volumes corresponded to cells having 2x, 4x, 8x, 16x, 32x, 64x, and 128x constitutions Similar findings by Geitler (1937, 1939, 1941) in the salivary glands of the water insect *Gerris lateralis* were reported previously (Section 9 1)

17 3 1 Asynapsis and Desynapsis

Both these processes represent asynaptic mutations and lead to the reduction or loss of synapsis However while in **asynapsis**, the homologous chromosomes either fail to pair completely during zygotene or pair very incompletely in **desynapsis** the homologues pair initially but fall apart during early diplotene or just after it but usually before metaphase I The asynaptic condition caused by major genes can be of varied origin

1 asynapsis owing to gene mutations
2 asynapsis in progenies of varietal or species hybrids
3 asynapsis owing to loss or addition of a chromosome or chromosome pair

Futhermore asynapsis can be induced or influenced by external environmental conditions especially by mutagens The same genetic and environmental influences are valid for desynapsis

Beadle (1930 1933b) discovered a gene in maize that upsets synaptic pairing He called this gene *asynaptic* (*as* chrom 1 53) The effect of this gene was that most of the chromosomes did not pair during zygotene and as a result occurred as univalents rather than bivalents at metaphase I Normal synapsis and crossing over are guaranteed only if two doses of *As* are present (*As As*) (Baker and Morgan 1969 Nel 1973) Evidence for genetic control of asynapsis was also discovered in *Matthiola* (Philip and Huskins 1931) *Drosophila* (Gowen 1933) *Datura* (Bergner et al 1934) peas (Koller 1938) *Oenothera* (Catcheside 1939) wheat (Smith 1939) rye (Prakken 1943) tomato (Soost 1951) rice (Katayama 1961) maize (Miller 1963 Sreenath and Sinha 1968) sorghum (Stephens and Schertz 1965) broad bean (Sjödin 1970) rape (Stringham 1970) cotton (Weaver 1971) and *Lolium* (Omara and Hayward 1978)

The structure that spans the region between two synapsed chromosomes is the **synaptonemal complex** (see Section 7 2 2 1) This complex ultrastructure is completely lacking in a *Drosophila* mutant in which crossing over in homozygous females is practically eliminated or almost completely reduced in the entire chromosome complement The name of that mutant is *c(3)G* ^{7} (chrom 3 57 4) (Meyer 1964 Smith and King 1968) Such a lack of synaptonemal complexes as well as the lack of pairing of chromosomes in pachytene may be a critical criterion for distinguishing between asynapsis and desynapsis Desynapsis is controlled by *ds* genes that lower the chiasma frequency or prevent chiasma formation entirely Both *as* and *ds* genes are similar in their action They both cause disturbances in micro- and megasporogenesis A third group of genes that also affect fertility in higher plants the *ms* genes are only effective in microsporogenesis (Section 17 4)

Desynapsis is a widespread phenomenon in the mutant collections of countless plant species

Desynaptic mutants have been reported in *Crepis* (Richardson 1935) wheat (Li et al 1945 Bozzini and Martini 1971) peas (Gottschalk and Jahn 1964 Gottschalk and Baquar 1971) sorghum (Magoon et al 1961 Sadasivaiah and Magoon 1965) oats (Thomas and Rajhathy 1966) rice (Misra and Shastry 1969) *Lolium* (Ahloawalia 1969) cabbage (Konvicka and Gottschalk 1971, Gottschalk and Konvička 1972) soybeans (Palmer 1974) *Allium* (Gohil and Koul 1971 Kaul

1975) barley (Scheuring et al 1976) and *Pennisetum* (Singh et al 1977 Koduru and Rao 1978 Rao and Koruru 1978)

Evidence of probably genetically determined asynapsis comes from the study of an azoospermic but otherwise healthy and normally developed man (Chaganti and German 1974) Pachytene pairing and chiasma formation at diakinesis were disturbed Univalents were observed in diakinesis No chiasmata were seen Almost all spermatocytes were in pachytene The patient s mother s brother and mother s sister s son were also infertile

17 3 2 Variation in Crossing Over

Beadle (1933b) assumed that crossing over in the asynaptic mutant of maize would be greatly reduced because there was very little chromosome pairing in zygotene as manifested in pachytene But Rhoades (1947) could demonstrate that the frequency of crossing over and of double crossing over in particular was much higher than normal in this mutant For instance in the ws_1-*lg*-*gl* region of chromosome 2 (*white sheath* ws_1 0 *liguleless* *lg*, 11 *glossy* *gl* 30), double crossing over was increased 25 times The same observation was made for the C_1-sh_1-*wx* region of chromosome 9 (*aleurone color*, C_1 26, *shrunken* endosperm, sh_1 29, *waxy* endosperm, *wx* 59) If chromosomes do not pair in zygotene, one could speculate that pairing and crossing over could happen during premeiotic cell divisions

Premeiotic crossing over was assumed for male *Drosophila* for which crossing over does not seem to occur during meiosis in the primary spermatocytes (see Section 8 2 1), but somatic crossing over does occur in the spermatogonia (Whittinghill 1937, 1947) Other meiotic mutants that reduce and/or change the distribution of crossing over in *Drosophila* can be classified into three categories

1 Reduction of crossing over without changes in the distribution pattern [An example is mutant *mei* 9 (Baker and Carpenter, 1972, Carpenter and Sandler, 1974)]
2 Reduction of crossing over with changes in the distribution pattern [Mutants involved are *mei*-41, *mei*-218, *mei*-251, *mei* S282, *mei* B, *a b o* and *mei*-68^{LV}(Bridges, 1929, Lindsley et al , 1968, Baker and Carpenter, 1972, Lindsley and Peacock, 1976, Valentin, 1973, Carpenter and Sandler, 1974 Parry, 1973)]
3 No reduction of crossing over but changes in the distribution pattern [An example is *mutant mei-352 (Baker and Carpenter, 1972)*]

All of these genes reduce crossing over when they are homozygous recessive in females If genes $c(3)G^{17}$ or $c(3)G^{31}$ are homozygous recessive, crossing over is almost completely absent (Carlson, 1972, Hall, 1972) But, when they are heterozygous, they show a nonuniform increase in crossing over (Hinton, 1966, Hall, 1972)

In a homozygous recessive mutant for gene *rec-1* of *Neurospora crassa* crossing over in the *his-1* locus some distance away is increased ten-fold above normal (Catcheside, 1977) As pointed out earlier, chiasmata are the visible evidence for meiotic crossing over (see Section 4 2 1) Consequently, the frequency and distribution of chiasmata in meiotic mutants have been used as a possible indicator for crossover disturbances In a recessive rye mutant investigated by Prakken (1943), pachytene pairing was almost normal but the total number of chiasmata was

reduced from an average of 12 6 to a range from 2 6 to 6 4 The distribution of chiasmata shifted toward the distal ends of the chromosomes Other mutants in which the total number of chiasmata was reduced were reported in *Crepis* and broad bean (Richardson 1935) and in tomato (Soost, 1951) Decreasing chiasma frequency was observed in inbred lines of rye as homozygosity increased (Lamm, 1936 Müntzing and Adkik, 1948) Similar results from inbreeding were obtained in maize (Blanco, 1948) and *Drosophila* (Blanco and Mariano, 1953) However, an inbred line of mice showed a higher frequency of chiasmata (Shizinsky, 1955) In a broad bean mutant, Sjödin (1970) found a change in distribution of the chias mata Similar observations were made in maize (Beadle, 1930, 1933b, Miller, 1963) peas (Koller, 1938 Klein, 1969) *Oenothera* (Catcheside, 1939), wheat (Li et al 1945) tomatoes (Moens, 1969a), and Scotch pine, *Pinus sylvestris* (Runquist 1968) Failure of chiasma formation in one particular chromosome (chromosome IV) was observed in *Hypocheris radicata* (Parker, 1975)
Meiotic mutants in humans are difficult to discover since pedigree data are harder to obtain But patients with Down s syndrome (see Section 16 2 4 1) have been reported to have an increased number of chiasmata per cell (Hulten and Lindsten 1973) This obviously is not necessarily a result of aneuploidy, since human XYY trisomics and aneuploids with extra unidentified small centric chromosomes have normal chiasmata counts (Evans et al , 1970, Hulten, 1970, Hultén and Lindsten, 1973)

17 3 3 Variation in Chromosome Size

Evidence that the size of chromosomes must be under genetic control was given by Thomas (1936) in perennial ryegrass and by Lamm (1936) in rye lines derived from inbreeding In the garden stock *Matthiola incana* a mutant exists in which the chromosomes are long while in the normal forms they are short (Lesley and Frost 1927) A reverse situation was discovered in sweet pea where the mutant form showed short chromosomes while the normal situation was characterized by long chromosomes (Upcott, 1937) Moh and Nilan (1954) discovered a meiotic mutant in barley *(sc)* that was characterized by extremely condensed diakinesis chromosomes In addition, the mutant had relatively well spread pachytene chromosomes that proved to be favorable for **pachytene analysis** (see Section 2 2) (Blickenstaff et al , 1958) A gene for long chromosomes was found in barley (Burnham 1946 McLennan, 1947, McLennan and Burnham, 1948)

17 3 4 Variation in Spindle Formation

Clark (1940) discovered a meiotic mutant in maize that is called *divergent spindle* (*dv* location unknown) When this gene is homozygous recessive (*dv dv*), the spindle fiber apparatus in meiosis I cells forms parallel or divergent fibers instead of those converging to the two poles The result is that the chromosomes fail to gather at the poles, but individual chromosomes or smaller chromosome groups come together and form separate nuclei The spindles at the second meiotic division may again be divergent Consequently, there may be more than four spores

Fig 17 6 Multiple microsporocytes (syncytes) in barley caused by a recessive gene Multiple sporocyte with 113 bivalents and 4 quadrivalents (From Smith 1942 Reprinted by permission of Burlington Free Press Burlington Vermont)

at the quartet stage (Fig 7 27), and 42% to 95% of the microspores are multinucleate A divergent spindle mutant was also discovered in crested wheatgrass (Tai, 1970)

Similar divergent spindles were found in a mutant that formed multiple sporocytes in barley (2n = 14) (Smith, 1942) Meiocytes were found with 14, 21, 28, 56, 112, and higher numbers of chromosome pairs Such meiocytes are also referred to as *syncytes*[1] (Levan, 1942b), which are formed by *cytomixis*[2] (Gates, 1911) Fusion of some of the chromosomes was thought to have taken place prior to meiosis, since in many cases multivalents had formed during synapsis Cell walls seemed to be absent in these so-called cells and sometimes all, or at least part, of the contents of an entire anther locule were included in one syncyte Some of the chromosomes seemed to have fused as late as metaphase I Very long metaphase plates were the result of this phenomenon (Fig 17 6)

Multiple spindle formation was reported in *Clarkia* (Vasek, 1962) About half of all meiocytes possessed two complete spindles at metaphase I

17.3 5 Other Variations in Meiosis

In a mutant of *Datura* (2n = 24) called *diad* (*d*), which resulted from pollen treatment with radium no second meiotic division occurred (Satina and Blakeslee, 1935) At the end of telophase I, the normally short period of interkinesis (see

[1] *Syncyte*–a polyploid or multinucleate cell formed usually by inhibition of cytokinesis, it leads to the formation of a *syncytium* (Haeckel 1894) which is a mass of protoplasm lodging many nuclei not separated by cell membrane

[2] *Cytomixis*–the fusion of the chromatin of two or more cells

Section 7.5) was replaced by a prolonged regular interphase during which a post-meiotic chromosome replication took place. Mitosis in the first male gametophyte division produced diploid nuclei that became diploid gametes.

Precocious chromosome division during meiosis I occurred in three mutants in tomato (Lamm, 1944; Clayberg, 1959) and *Alopecurus* (Johnson, 1944). Normally chromatids do not separate until anaphase II. In the mutants the chromatids separated during anaphase I, telophase I, or interkinesis. If a metaphase II plate was formed, the chromatids moved to the opposite poles at random.

Another group of meiotic mutants are those that result in frequent chromosome breakage. In some of these, bridges and fragments have often been observed at anaphases I and II. These mutants were demonstrated to occur in peas (Klein, 1969; Klein and Baquar, 1972). Plants with high frequencies of anaphase bridges and fragments without clear evidence of mutant origin have been observed in *Scilla* (Rees, 1952, 1958), *Solanum* (Lamm, 1945), *Picea* (Anderson, 1947), *Matricaria*, *Hyoscyamus* (Vaarama, 1950), *Sorghum* (Magoon et al., 1961), *Allium* (Koul, 1962), and *Podophyllum* (Newman, 1967). Other mutants that are often associated with chromosome breakage during meiosis are the *sticky* mutants. The first one discovered was mentioned previously in connection with chromosome breakage during mitosis (Section 17.2). This was the *sticky gene in maize* discovered by Beadle. Adherence of the chromosomes at anaphase I, and clumping, and sometimes breakage were characteristics that were associated with this stickiness. With this study Beadle could demonstrate that genes affecting chromosome behavior segregate and recombine in the same fashion as those that influence the morphology of plants or animals. Other sticky meiotic mutants sometimes associated with chromosome breakage were reported in *Alopecurus* (Johnson, 1944), durum wheat (Martini and Bozzini, 1965), broad bean, tomato (Moens, 1969a), *Brassica* (Stringham, 1970), *Collinsia* (Mehra and Rai, 1970, 1972), and peas (Klein, 1971).

17.4 Male Sterility

Aberrant meiotic behavior caused by nuclear genes, as discussed in Sections 17.3, can be the reason for genetically determined male sterility. Other phenomena associated with male sterility can be, for instance, pollen abortion, failure of anther dehiscence, anther abortion and distortion, and pistillody of the anthers (the metamorphosis of anthers into pistils) (Gottschalk and Kaul, 1974). Microsporogenesis *is really the rectum of disturbances and not megasporogenesis*. The relatively unprotected haploid male gametophyte is probably more vulnerable than the protected embryo sac (Swanson, 1957). More than 400 *ms* mutants in over 50 species have been reported so far (Gottschalk and Kaul, 1974). In most of the male sterility mutants in maize, abortion occurred after meiosis, but, in a few, male sterility was caused by aberrant meiotic behavior (Beadle, 1932b). For instance, male gametophyte development in genetic male steriles ms_3, ms_7, and ms_{11} breaks down between the fifth and tenth days after meiosis (Madjolelo et al., 1966). Most male sterility genes are recessive (*ms ms*). One of them in maize is a dominant gene

(Ms_2) Twenty different male sterility genes in maize had been described by 1935 (Emerson et al 1935) The numbering system indicates 43 by 1979 (Golubovskaya 1979) Some of the maize male sterility genes are *ms* (Chrom 6 17) *ms* (Chrom 9 67) ms_1 (Chrom 8 14) ms_9 (Chrom 10) *ms* (Chrom 1 23) and *ms si* [Chrom 6 (19)]

Genetic male sterility in plants has not occurred widely in nature but it has been screened for in almost all crops Besides in maize genetic male sterility has been found for instance in barley (Suneson 1940 Hockett and Eslick 1971) tomato (Rick 1948) lima bean (Allard 1953) potato (Okuno 1952) and cotton (Richmond and Kohel 1961)

Genetic male sterility is of great value to plant breeders It is increasingly being used for the production of hybrid varieties Marker genes closely linked with the male sterility genes make roguing of the male fertile plants easier since the progeny of a cross between a male sterile plant (*ms ms*) and a male fertile plant (*Ms ms*) always produces 50% undesired male fertiles (*Ms ms*) In some instances male fertile plants can be identified before pollen shedding and can be removed In watermelon a sterile homozygous recessive male sterile mutant has as a marker *glabrous leaves* which allows the nonmarked male fertiles to be removed (Watts 1962) In lettuce male steriles (*ms ms* ms_2 ms_2 *ms ms*) have narrow sharply cut leaves that can be recognized prior to flowering (Lindqvist 1960)

17 5 Preferential Segregation of Chromosomes

Meiotic segregation of chromosomes toward the poles at anaphase I is generally at random (see Section 7 3) But such behavior is difficult to prove unless the two chromosomes of a bivalent are morphologically marked or unless there is genetic proof of nonrandom segregation of genes that are on different chromosomes The randomness of chromosome segregation was proved in several grasshopper species in which heteromorphic homologous chromosome pairs could be studied (Carothers 1917 1921)

Nonrandomness of chromosome segregation in meiosis has been observed in several cases has been called **preferential segregation**, and leads to **segregation distortion** (Sandler and Hiraizumi 1961) An instance in which preferential segregation was proved both by a marker chromosome as well as by distortion of genetic ratios was observed in maize An abnormal chromosome 10 was discovered by Longley (1938) in certain maize strains grown by North American Indians This chromosome has a largely heterochromatic knobbed segment added to the long arm of chromosome 10 Burnham (1962) noticed that this terminal segment superficially resembles the well known B chromosome of maize In plants that are heterozygous for abnormal 10 70% of the megaspores carry this distinctive marker chromosome (Rhoades 1942 1952) This preferential movement has been explained as a purely chromosome related phenomenon The abnormal 10 chromosome has a tendency to form a neocentromere (see Section 2 1 2) The spindle fiber instead of attaching to the normal position at the primary constriction of chromosome 10 pulls this chromosome at a new location close to the

abnormally knobbed end This movement is precocious in that abnormal 10 passes quickly to the nearest pole before the other chromosomes normally separate at anaphase This movement is thought to cause the abnormal 10 to be preferentially distributed to the basal megaspore that, in the linear quartet (see Fig 8 7), becomes the functional embryo sac Not only the abnormal 10 segregates preferentially, but other chromosomes that possess knobs also can segregate nonrandomly in the heterozygous presence of one abnormal 10 (Longley, 1945) The knobs of these chromosomes are activated by the presence of abnormal 10 and become neocentromeres that move precociously in anaphase Tests were conducted involving genes closest to the knob on the short arm of chromosome 9 In the heterozygote, the knobbed chromosome carried the alleles for *aleurone color* (C_1 26), *shrunken endosperm* (sh_1 29), and *waxy endosperm* (*wx* 59) The knob had a near *0* position on the chromosome map The homologous chromosome *without the knob carried the alleles* c_1, SH_1, *and Wx From these map data, it* can be seen that C_1 (26) was closest to the distal knob and *wx* (59) closest to the centromere (see genetic maize map, Fig 4 8) C_1 showed the greatest percentage of preferential segregation, sh_1 was similar in this respect, but *wx* showed very little Preferential segregation is increased for loci that are involved in frequent crossing over between the centromere and the knob and are in close proximity to this distal knob This relationship is best illustrated in Fig 17 7 Preferential segregation in translocation heterozygotes has already been discussed (see Section 14 3)

Another mechanism involving preferential segregation is often referred to as **meiotic drive.** This was defined by Sandler and Novitski (1957) as a meiotic phenomenon that modifies the breeding structure from the expected ratios by changing the frequencies of alleles in a population An example for this phenomenon was

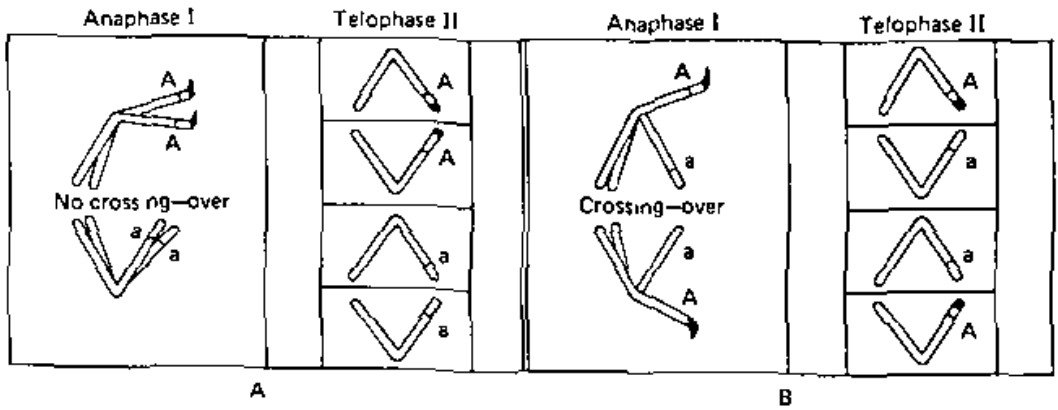


Fig 17 7A and B Preferential segregation caused by neocentric activity of the knobbed abnormal 10 chromosome in maize (*A*) During meiosis in the embryo mother cell the neocentromere affects the knob orientation (gene A) by pulling the chromatid on which it is located to the extremity of the AI pole When no crossing over occurs one chromosome carries the neocentromere and the other does not Preferential segregation is negligible (*B*) *When crossing over occurs, both chromosomes have* one neocentric and one normal chromatid The neocentric chromatids move to the extremes of the AI pole and are preferentially included in the terminal (functional) quartet cell during A II (From Sybenga 1972 Redrawn by permission of Elsevier/North-Holland Biomedical Press, Amsterdam)

orginally thought to be the gene for *Segregation Distorter* in *Drosophila* (*SD* chrom 2 55 0 Centromere location) This gene is located close to the heterochromatic-euchromatic junction and according to one interpretation may involve chromosome breakage in that region (Sandler and Novitski, 1957) Similar alleles of this gene have been found by other workers (Mange, 1961, Greenberg, 1962) The females (*SD SD* or *SD sd*) of this mutant behave normally The homozygous dominant males (*SD SD*) are sterile The heterozygous male (*SD sd*) produces a majority of *SD*-bearing functional sperm often more than 95% Generally, one would expect a 1 1 ratio of *SD*- to *sd*-bearing sperms

The earliest explanation of this phenomenon was the **dysfunctional sperm hypothesis** of Sandler et al (1959) According to this hypothesis the *sd* bearing chromosome (second *Drosophila* chromosome) through the action of *SD* breaks at a specific location, that after chromosome replication is subject to reversed sister strand reunion (see Section 11 1) causing chromatid bridges and death or non function of the cells they tie together The cytological evidence for this hypothesis could not be verified subsequently

An alternative to this hypothesis was the **functional pole hypothesis** of Peacock and Erickson (1965) that states

a that *Drosophila melanogaster* normally produces two functional and two nonfunctional sperms from each spermatocyte
b that the *sd* bearing chromosome is directed by *SD* to the nonfunctional cell pole during spermatocyte division

The functional pole hypothesis was discredited by Hartl et al , (1967) and independently by Nicoletti et al , (1967) They established that the number of offspring produced by *SD sd* males was about half as great as that produced by normal *sd sd* males A condition for the functional pole hypothesis would be that the *SD sd* males will produce no less functional sperm than normal males More recent thought again favors the dysfunctional sperm hypothesis *SD* could induce some kind of physical alteration in *sd* too small to be detected cytologically, and by this action render *sd* nonfunctional (Hartl and Hiraizumi, 1976)

Another gene was discovered that seems to have a regulating function in regard to *SD* It occurs together with *SD* and is called *Stabilizer of Segregation Distorter* (*St-SD* Chrom 2 close to *bw* 104 5) In the absence of *St* the activity of *SD* is more variable (Sandler and Hiraizumi, 1960) Hartl and Hiraizumi (1976) believe that the action of *St* appears to be really a cumulative effect of minor modifier genes

Zimmering et al (1970) have suggested five possible mechanisms of unequal transmission of homologues

1 a supplementary replication of the meiotically driven chromosome and the associated loss or degeneration of its homologue
2 impairment of sperm function by abnormal chromosome behavior (e g , chromosome breakage) with the sperm that carries the favored chromosome remaining unimpaired
3 sperm competition or malfunction, depending on the genetic constitution of an organism
4 preferential segregation of a favored homologue to the functional pole at meiosis
5 differential acceptance of two different kinds of sperm by the ovum

Genetic proof for nonrandom segregation sometimes leads to the conclusion that preferential chromosome segregation is involved Such genetic segregation was

observed in the progeny of crosses between laboratory stocks of mice and was referred to as **genetic affinity** (Michie, 1953, 1955, Parsons, 1959, Wallace, 1953, 1957, 1958, 1959, 1961) It was found that genes located on different chromosomes *(V and XIII) tended to segregate together at meiosis and pass to the same pole* This results in genetic linkage between genes of nonhomologous chromosomes Similar interdependence between chromosomes in their movement toward the pole was reported for crane fly spermatocytes (Forer and Koch, 1973)

Chapter 18
Variation in Function of Sex Chromosomes

The so-called "normal function" of the sex chromosomes was discussed in Chapter 5 As there are genetic factors that upset the normal function of the autosomes, there are naturally also those that upset the normal function of sex chromsomes Since the sex chromosomes constitute a distinct group with a much specialized task of specific genetic expression their special treatment is warranted and is presented in this chapter

18.1 Variation in Sex Ratio

The normal sex ratio is determined by the number of males vs females at birth, which generally is 1 1 or close to it Deviations from this ratio can be caused by several factors Sturtevant and Dobzhansky (1936) discovered a sex ratio gene in *Drosophila pseudoobscura* and *D persimilis* This gene caused abnormal frequency of daughters (more than 90%) if it was present in the male parent of a cross During the first meiotic division of the spermatocyte the X and Y chromosomes did not pair but the X chromosome split twice and separated mitotically (Fig 18 1) during meiosis I so that each daughter cell received one X chromosome The Y chromosome did not divide during meiosis I but passed to one of the first division poles, became enclosed in a vesicle, and degenerated Since the X chromosome had split twice during meiosis I (probably two replications during premeiotic interphase), it could again separate in meiosis II, distributing one X chromosome to each sperm Novitski et al (1965) and Polansky and Ellison (1970) reinvestigated the *sex ratio* gene in *D pseudoobscura* and found that the mechanism was different During anaphase I the X and Y chromosomes regularly passed to opposite cell poles Following meiosis I the Y chromosome degenerated leading to nonfunctional sperm formation Consequently, each primary spermatocyte produced only two instead of four functional sperms Thus, the X chromosome did not split twice as was suggested by Sturtevant and Dobzhansky

Another case of the distortion of the sex ratio in favor of females was reported by Novitski and Hanks (1961) and Erickson and Hanks (1961) Males of *Drosophila melanogaster* containing the gene *Recovery Disrupter* [*RD[1]*, chrom 1 62 9] may produce approximately 67% female progeny, due to a reduction in the recovery of the Y chromosome The mechanism involved causes a fragmentation of the

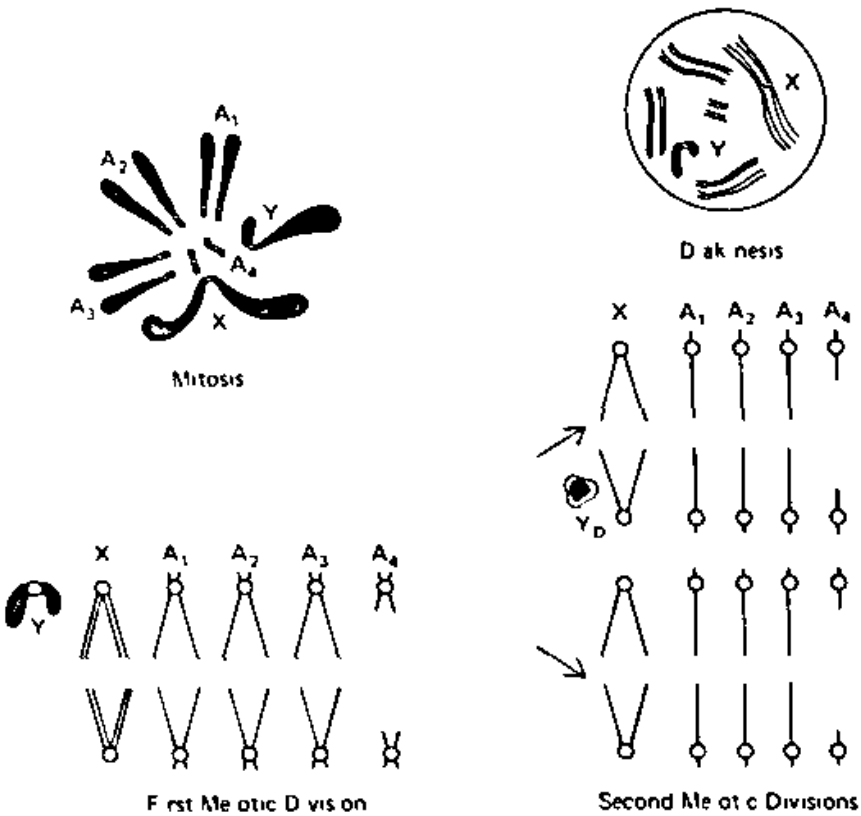


Fig 18 1 Meiosis in a "sex ratio" male of *Drosophila pseudoobscura* (A_{1-4} = autosomes) According to an older concept, the X splits in both meiotic divisions The Y is heteropycnotic and eventually disintegates (Y_D) (From White 1954 After Sturtevant and Dobzhansky 1936 Redrawn by permission of the University Press, Cambridge)

Y chromosome during meiosis (Erickson, 1965) A second *RD* chromosome [*RD(2)*] has been discovered on chromosome 2, but the map location has not been determined (Wallace in Lindsley and Grell, 1968) This factor is thought to be another example of *meiotic drive* (see Section 17 5)

18 2 Different Sex Chromosome Systems

In Chapter 5 the basic form of sex determination in animals and in some plants was discussed In the basic system one sex has a pair of chromosomes that, microscopically, are similar (XX) and the other sex has visibly different chromosomes (XY) The closest deviation from this XY XX system is the XO XX system The O in this formula merely indicates the absence of the Y chromosome As mentioned, Henking in 1891 found the first one of such systems in the insect *Pyrrhocoris apterus* (Chapter 1) In meiosis I of such XO organisms, the X chromosome oriented itself in the metaphase plate without forming a bivalent Usually, if autosomal univalents occur in meiosis, they do not line up on the metaphase I plate Here, the X chromosome moves randomly to one of the two cell poles and later is recovered in 50% of the gametes Autosomal univalents often lag behind in ana-

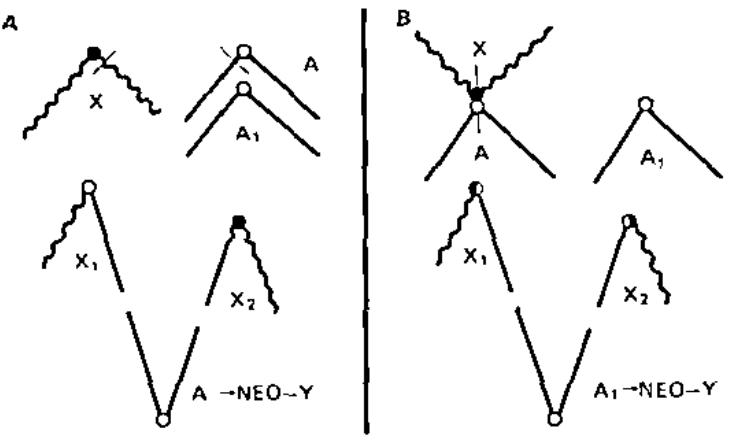


Fig 18 2A and B Two possible origins of multiple sex chromosome systems (*A*) A reciprocal translocation occurs between the X chromosome of an XO XX system and one of a pair of autosomes (A of AA_1) The two new translocated chromosomes become X chromosomes (X_1X_2) The second autosome (A_1) becomes the neo-Y chromosome (*B*) The X and the A chromosome fuse at the centromere (centric fusion). The centromere then divides transversely instead of longitudinally resulting in two new X chromosomes each possessing one arm of the old X and one arm of A (X_1, X_2) (From Hughes Schrader, 1950 Redrawn by permission of Prentice Hall Inc, Englewood Cliffs, New Jersey)

phase and do not always get included in the daughter nuclei A great majority of species in the insects of the Orthoptera and Odonata have the XO XX system (White, 1973)

Bivalent pairing and chiasma formation in meiosis I apparently are not always necessary requirements for chromosome distribution, as is the case for autosomes For instance, in some Tipuloidea species, the X and Y chromosomes do not synapse but distribute regularly to the poles in meiosis I (Wolfe, 1941) Sometimes the sex chromosomes form a very brief end-to-end association in diakinesis as in the hemipter *Rhytidolomia senilis* (Schrader, 1940b) This transitory chromosome association was called **touch-and-go pairing** by Wilson in 1925

Translocations between one of the sex chromosomes and an autosome can lead to multiple sex chromosome systems If in the XO XX system, the X in the XO sex translocates with one of the autosomes, an X_1X_2Y condition can arise (Fig 18 2A, B) The two translocated chromosomes will become X_1 and X_2 while the non-translocated homologue of the autosome pair involved (A_1) becomes the neo-Y chromosome Such sex chromosome trivalents (X_1X_2Y) have been observed in the mantids (Hughes-Schrader, 1950) The same phenomenon has been observed in 14 genera of grasshoppers (Helwig, 1941, 1942) In such a system the autosomal neo-Y chromosome becomes confined to the male sex The female has one more chromosome than the male (X_1X_2Y $X_1X_1X_2X_2$ system) Other examples for this system are *Drosophila miranda* (MacKnight and Cooper, 1944) and the Rhodesian pygmy mouse (Matthey, 1965)

Another possbility is a translocation between the Y chromosome and an autosome, which leads to the XY_1Y_2 condition In such a system the male has one more chro-

mosome than the female (XY_1Y_2 XX system) Examples for this system are *Drosophila americana* (Spencer, 1940), the gerbil (Wahrman and Zahavi, 1955), and some bats (Baker and Hsu, 1970)

Complex systems of higher magnitude can be based on the XO XX or XY XX systems $X_1X_2X_3X_4O$ males occur in the aphid *Euceraphis betulae* (Shinji, 1931) All X chromosomes pass to the same pole during meiosis I, and the secondary spermatocytes that do not have any X chromosomes degenerate Only one type of sperm is formed The scale insect, *Matsucoccus gallicola*, even has six X chromosomes in the male ($X_1X_2X_3X_4X_5X_6O$, Hughes-Schrader, 1948)

Complex systems based on the XY XX scheme have been found in the beetles of the genus *Blaps B walti B mortisage* and *B mucronata* had $X_1X_2X_3Y$ (Nonidez, 1915, Guenin, 1949, Lewis and John, 1957), *B gigas* had $X_1X_2X_3X_4Y$ (Guenin, 1949), and the most complex sex chromosome system known is recorded for *B polychresta*—$X_1X_2X_3X_4X_5X_6X_7X_8X_9X_{10}X_{11}X_{12}Y_1Y_2Y_3Y_4Y_5Y_6$ (Guénin, 1953)

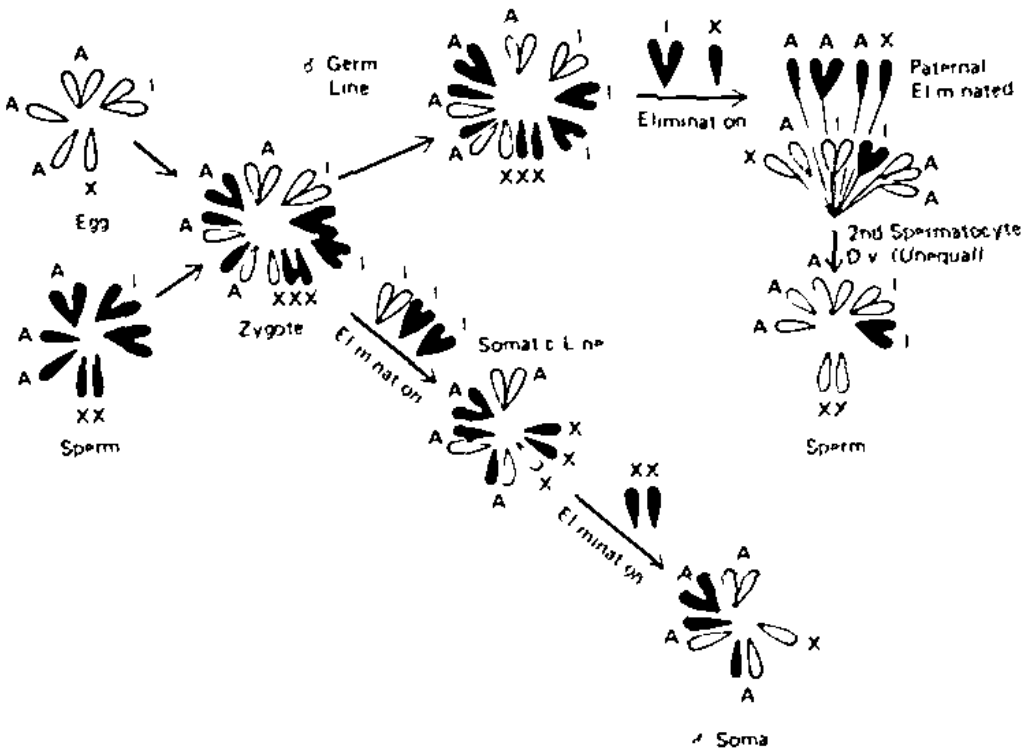
18.3 Cytogenetics of *Sciara*

Striking meiotic anomalies with interesting departure in sex determination have been observed in the fungus gnats, small two-winged flies of the family Sciardidae feeding on fungi The thorough studies of the species *Sciara coprophila* are fairly representative of the chromosome mechanism in the genus *Sciara* in general (Metz, 1931, 1933 1934 1936 1938a, 1938b, Metz and Schmuck, 1929, 1931, Schmuck and Metz, 1932)

Sciara has a basic complement of four homologous chromosome pairs, three autosome pairs, and one X pair (6A+2X, ♀ soma) But in the germ line there are, in addition, one to several **limited chromosomes** (l) that are limited to the germ line During the growth of the nurse cells and of the primary spermatocytes, these chromosomes are **positively heteropycnotic** (see Section 2 2 1) in that they are compact and tightly coiled and darker appearing than the basic set They are also larger in length and diameter and apparently without specific genetic activity (Fig 18 3) The meiosis of the females is normal Bivalents are formed and genetic evidence for crossing over exists (Schmuck and Metz, 1932) Spermatogenesis, however, is very irregular (Metz et al , 1926, Metz, 1933) The homologous chromosomes do not pair during meiotic prophase I, and the chromosomes remain univalents

The chromosome complement in the male germline initially consists of 3A[II]* 3X +3l+ (Fig 18 3) Before the first spermatocyte division, an X chromosome and an occasional superfluous l-chromosome become eliminated The first meiotic division spindle is a **monaster** that performs a **monocentric mitosis.** The maternal chromosomes (white in Fig 18 3) become attached to the spindle and move to a single pole This fact is based on genetic as well as on cytological evidence (Metz, 1938a) The paternal chromosomes, in spite of also being attached to the mon-

*3A[II] = 3 pairs of autosomes



CYTOGENETICS OF SCIARA

Fig 18 3 Meiotic and mitotic anomalies with departure in sex determination in *Sciara coprophila* A–autosomes, 1–limited chromosomes, X–sex chromosomes, ⇝–maternal chromosomes, ➖–paternal chromosomes (Modified from Crouse, 1943 Redrawn by permission of the University of Missouri Agricultural Experiment Station, Columbia)

aster, back away from that pole and become collected into a tiny bud that later pinches off and becomes discarded The maternal and paternal (black and white in Fig 18 3) l-chromosome pass with the maternal A and X chromosomes into the secondary spermatocytes Since a paternal l-chromosome does not possess any specific genetic function, no paternal genes become included in the secondary spermatocyte

During the second spermatocyte division, the only remaining X chromosome *divides Both halves move precociously to the same pole (non-disjunction) so that* each sperm always possesses two X chromosomes, since the cells without X chromosomes degenerate

As mentioned, the somatic tissue (soma) from which the germ line branches off consists of three pairs of autosomes (3A''), 3 X chromosomes (3X) and one or more l-chromosomes (3 are shown in Fig 18 3) The l-chromosomes become discarded at the fifth or sixth cleavage division after zygote formation One or both paternal X chromosomes are eliminated shortly thereafter, during the seventh or eighth cleavage division This elimination will determine if the individual becomes a male or female If both paternal X chromosomes are eliminated, the soma becomes male (XO, Fig 18 3) If only one of the two paternal X chromosomes is elimi-

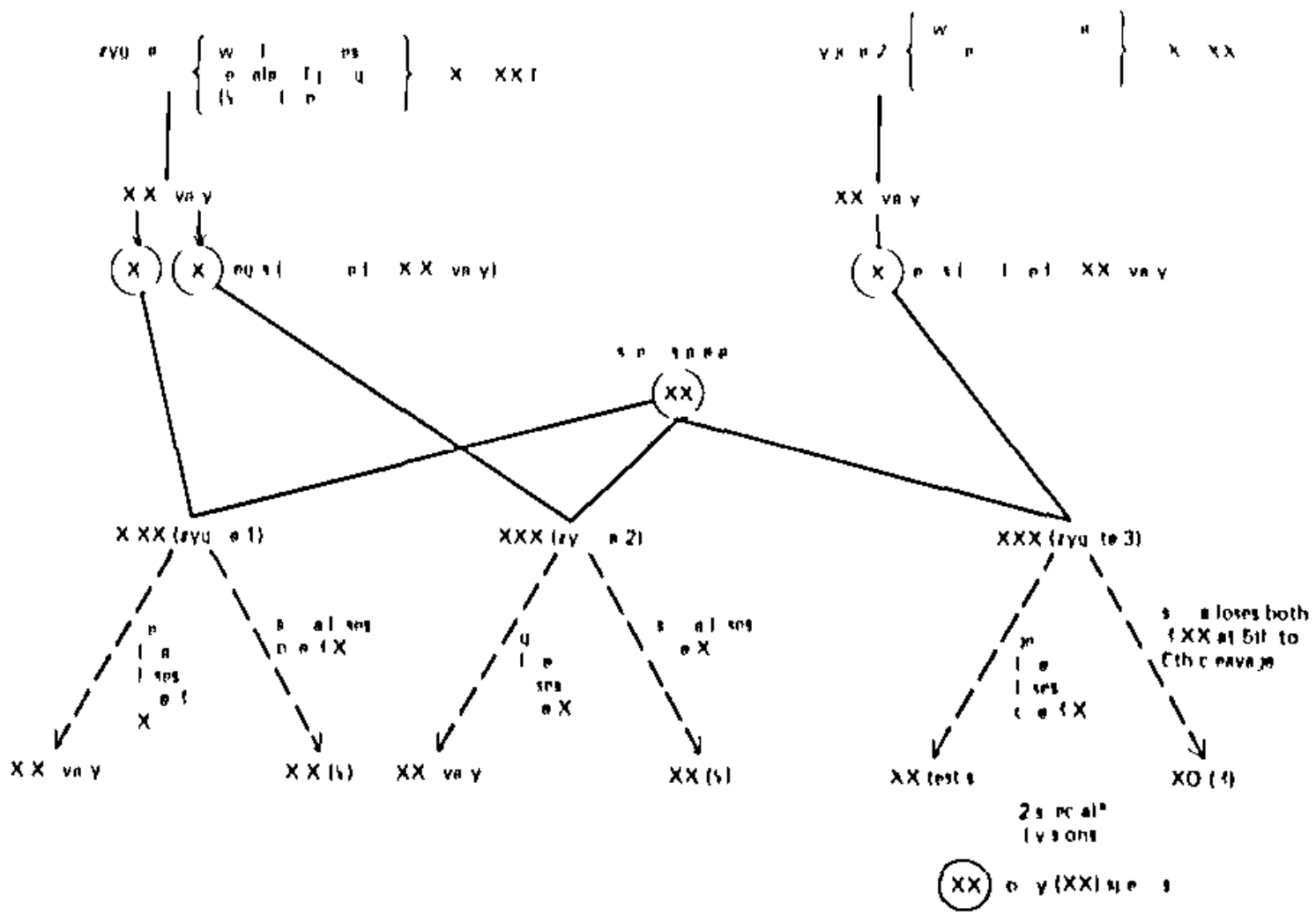


Fig. 194 Chromosome mechanism of sex determination in *Sciara coprophila* (After Metz 1938) From Barnham

nated, the soma becomes female (XX) Consequently, males and females in *Sciara* differ in their somatic chromosome number (*Sciara coprophila* ♂ 2n=7, ♀ 2n=8)

The **chromosome elimination** of the l-chromosomes and the X chromosomes in the soma is similar in nature The prophase is apparently normal Along with the normal chromosomes, the l chromosomes become attached to the mitotic spindle in metaphase and open out at the centromere but fail to separate at their distal ends As a result the l chromosomes remain in the equatorial plate and eventually degenerate They are not included in the daughter nuclei This elimination process may be closely associated with the heteropycnotic or heterochromatic nature of the l-chromosomes Heterochromatin has a tendency to become sticky under certain conditions, which may lead to the inability of these chromosomes to divide normally

In *Sciara* the genotype of the father has no influence on the sex of the progeny (Metz and Moses, 1928) In a normal XO XX system, there are two kinds of sperm produced, one with an X and one without In *Sciara* all sperm have 2X In *S coprophila* for instance, the offspring of any pair mating are all of one sex (**unisexual progenies**), either male or female The sex of the offspring consequently must depend on the genetic constitution of the mother Some mothers produce only male and some only female offspring There appararently are no visible cytological differences between these two kinds of mothers They must differ in an invisible genetic factor The X chromosome carrying this factor could be designated as X' Germ lines of female producing females would be heterozygous for this factor (X'X) and females producing males would be homozygous (XX) The heterozygous mothers (X'X) produce two kinds of eggs, X' and X If X' eggs are fertilized by XX sperm, X'XX zygotes will result that after elimination of one paternal X will become females (X'X) that, in turn, produce all female offspring (X'XX) The homozygous mothers (XX) produce only one kind of egg (X) If they become fertilized by XX sperm, they produce XXX zygotes that after elimination of one X become XX females that produce only male progeny (Fig 18 4)

Chapter 19
Apomixis and Parthenogenesis

Asexual reproduction occurs in plants as well as in animals In plants, asexual reproduction is commonly known as apomixis

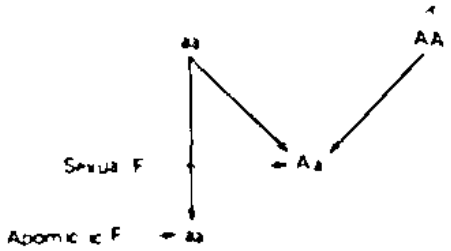
19 1 Apomixis in Plants

Apomixis is the replacement of sexual reproduction (**amphimixis**) by various types of asexual reproduction that do not result in the normal fusion of haploid gametes (Rieger et al 1976)

Apomicts can be obligate or facultative In an obligate apomict every plant of the species is always apomictic In facultative apomicts sexual and apomictic reproduction occurs in the same plant It is very difficult to classify a species as an obligate apomict because sexual or facultative apomicts may occur in nature that may not have been detected yet For instance, Young et al (1978) studied embryo sacs of formerly known obligatory apomict buffelgrass *(Cenchrus ciliaris)* and detected that about 10% of 1,300 pistils investigated showed single, fully differentiated, 8 nucleate embryo sacs that were indistinguishable from those of known sexual plants observed by the same method Aposporous and sexually appearing embryo sacs were observed within the same plant They concluded that this possibly indicates the presence of facultative apomixis in buffelgrass

Nevertheless some species have been classified as obligate apomicts For instance, almost all apomictic Compositae are obligate rather than facultative apomicts (Stebbins, personal communication) Other examples are *Cooperia* (Coe, 1953) and the American polyploids of *Crepis* (Stebbins and Jenkins, 1939) According to Stebbins many species of the Rosaceae are mainly facultative apomicts, while the Gramineae are equally divided into both groups Other examples of facultative apomicts are found among species of *Poa, Potentilla, Rubus Citrus* (Brown, 1972), and *Pilsella* (Rosenberg, 1917)

Apomixis can be implicated in a situation in which two phenotypically different parents when crossed result in an F_1 progeny that are all phenotypically like the homozygous recessive female parent In normal sexual reproduction, the progeny of such an F_1 cross should be phenotypically like the homozygous dominant pollen parent The following illustrates the two conditions



Additional evidence for apomixis is the maternal resemblance in the chromosome number after reciprocal crossing (Einset 1947)

Apomixis can be subdivided into **agamosperms** and **vegetative reproduction** In agamosperms there is seed formation but reproduction is asexual In vegetative reproduction there is no seed formation and the new individual forms from a group of differentiated or undifferentiated cells (Fig 19 1)

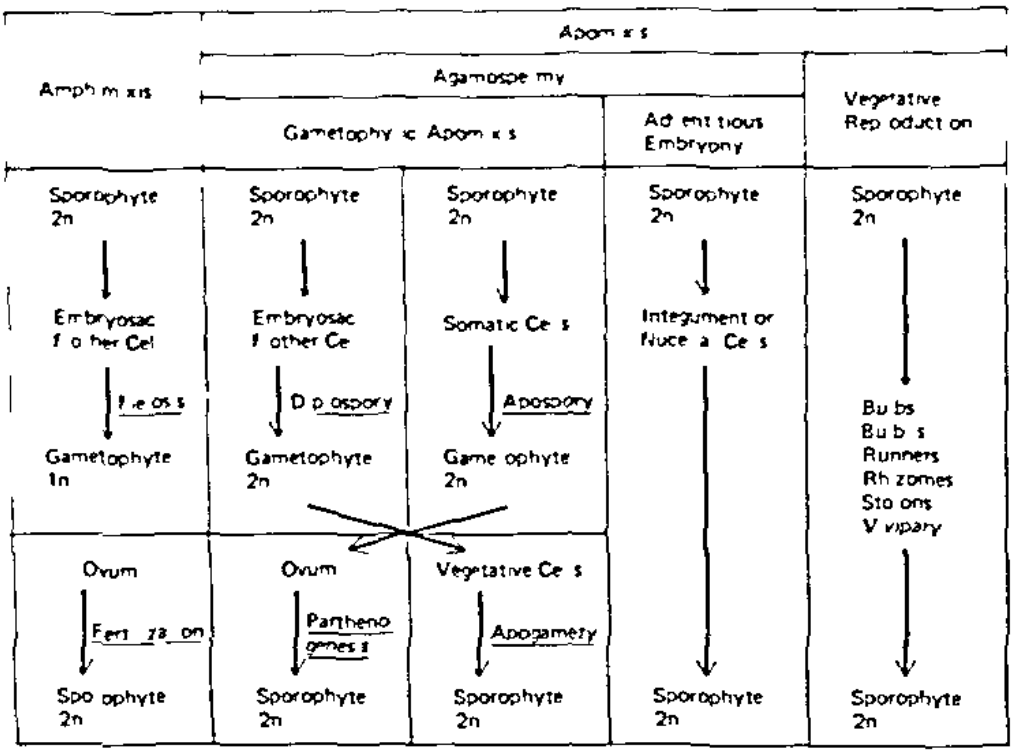


Fig 19 1 Diagrammatic representation showing the interrelationships of apomictic processes as compared to normal amphimixis (Modified after Stebbins 1950)

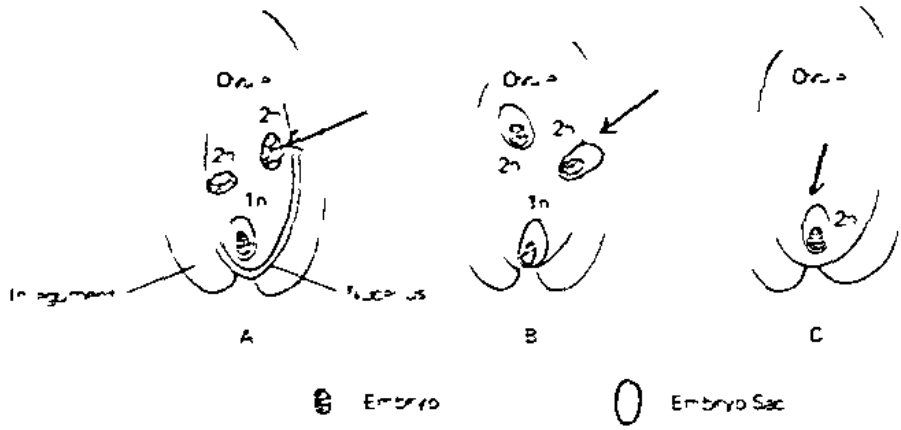


Fig. 19.2A–C Comparison of different forms of agamospermy. (*A*) Adventitious embryony: 1n, normal haploid embryo sac; 2n, two adventitious embryos. (*B*) Apospory: 1n–normal haploid embryo sac with sexually produced embryo; 2n, two diploid aposporous embryo sacs containing apomictic embryos. (*C*) Diplospory: 2n, an embryo sac containing an apomictic embryo. (Modified from Brown, 1972. Redrawn by permission of the C. V. Mosby Company, Saint Louis, Missouri.)

2. those that depend on endosperm development but not on fertilization, such as *Coelebogyne ilicifolia* (Schnarf, 1929)
3. those that depend neither on fertilization nor on endosperm development, such as the jointed cactus *Opuntia aurantiaca* (Archebald, 1939)

19.1.2 Somatic Apospory. This is one of the two forms of gametophytic apomixis. It means that the functional embryo sac does not develop from the megaspore but from a somatic cell. Two main types of somatic apospory were reported, the *Hieracium* type and the *Panicum* type.

The *Hieracium* type was found in some species of *Hieracium* (Rosenberg, 1906, 1907), *Artemisia* (Chiarugi, 1926), *Crepis* (Babcock and Stebbins, 1938), and in other genera. One or more somatic cells begin to enlarge, become vacuolate, and develop directly into the initial cell of the gametophyte. Three nuclear divisions result in the normal 8-nucleate embryo sac, but the nuclei have the *2n* somatic rather than the gametic chromosome number (n).

The *Panicum* type was reported for *Panicum maximum* (Warmke, 1954) and for other members of the Panicoideae (Emery, 1957, Emery and Brown, 1958, Simpson and Bashaw, 1969, Bashaw et al., 1970). One or more nucellar cells develop into an aposporous embryo sac. After vacuolation only two nuclear divisions result in a 4-nucleate embryo sac. All four nuclei remain in the micropylar region. Differentiation gives rise to two synergids, one egg and one polar nucleus, all having a somatic chromosome number (2n). Often more than one embryo sac forms in an ovule, but usually only one embryo sac functions in seed formation (Young et al., 1978).

19.1.3 Diplospory. In diplospory the embryo sac develops from an archespore cell, but meiosis is either missing or does not result in chromosome reduction. A meiosis that does not lead to chromosome reduction is called **apomeiosis** (Ren-

ner 1916) The reasons for failure of reduction can be lack of chromosome pairing and of chromosome contraction, retardation of meiosis, and precocious meiosis. Apomeiotic divisions can range from almost meiotic to typical mitotic. In *Erigeron karwinskianus* 80% of the megasporocytes form restitution nuclei in anaphase I (Battaglia 1950) The movement of the chromosomes in anaphase I is so erratic and scattered that by telophase I they are spread along the entire spindle (Fig 19 3) The nuclear envelope forms adjacent to these scattered chromosomes and eventually will enclose all chromosomes within one single restitution nucleus rather than within the normal two This leads to the unreduced chromosome number in some forms of diplospory

Apomeiosis can also be caused by asynapsis If the chromosomes do not pair, they remain as univalents and become enclosed in a diploid restitution nucleus. This system is typical for some species of *Taraxacum* A mitotic division follows after restitution and a dyad with the somatic chromosome number in each nucleus is formed The embryo sac develops from one of the two dyad cells (Schnarf, 1929 Rosenberg 1930 Gustafsson, 1932, 1935a 1935b 1937) Other examples of asynapsis with subsequent formation of two or four nuclei formed from divisions of the archesporial cell are *Artemisia*, *Eupatorium* and *Poa serotina*.

19 1 2 4 Pseudogamy Somatic apospory and diplospory are generally linked with **pseudogamy** (Focke, 1881) in that they require pollination This kind of pollination does not lead to fertilization of the egg but is necessary to stimulate embryo formation In pseudogamy a male gamete is necessary for embryo, endosperm and seed formation Pseudogamy has been reported in species of *Poa*, *Rubus Potentilla Ranunculus Hypericum Parthenium Citrus* and *Allium*. Species that do not show pseudogamy are found in *Hieracium*, *Taraxacum*, *Antennaria Crepis and Calamagrostis* (Gustafsson 1946 1947a 1947b) Some species can use pollen of related species for stimulation as was discovered for *Pennisetum setaceum* by Simpson and Bashaw (1969)

19 1 2 5 Parthenogenesis **Parthenogenesis** is the development of an embryo from an ovum without the participation of a sperm In parthenogenesis the embryo *always* develops from the female gamete or ovum In **apogamety** a non gametic 2n vegetative cell of the embryo sac (female gametophyte) produces an embryo (Fig 19 1) The normal life cycle of a seed plant (Angiospermae) consists of an alternation of 2n sporophyte and 1n gametophyte generations (diplohap-

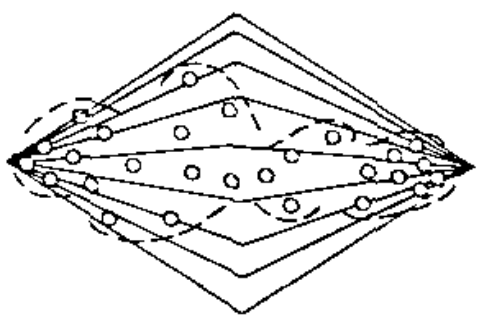


Fig 19 3 Apomeiosis leading to the formation of a **restitution nucleus** at telophase I The nuclear envelope (dashed line) forms around the scattered chromosomes and encloses all of them in a single restitution nucleus (From Brown 1972 Redrawn by permission of the C. V Mosby Company Saint Louis Missouri)

lonts, Chapter 8, introduction) These generations are separed by the events of meiosis and fertilization that alternately reduce and restore the somatic chromosome number (amphimixis, Fig 19 1) Agamospermy then is the by passing of meiosis and fertilization in the process of embryo and sporophyte formation
According to the embryological pathways chosen there exist four different avenues leading to the formation of agamospermous seed (Fig 19 1) These are

1 diplosporic parthenogenesis
2 diplosporic apogamety
3 aposporic parthenogenesis
4 aposporic apogamety

Here we are concerned with the two forms of parthenogenesis (1 and 3) Diplosporic parthenogenesis occurs in quite a few species of the Compositae and in many other families Grant (1971) gives a summary of the species in which it has been observed Aposporic parthenogenesis occurs in eight different species of *Crepis*, in numerous species of *Hieracium* in some Rosaceae and others

19 1 2 6 Apogamety As already mentioned in apogamety, a vegetative cell in the embryo sac produces an embryo Apogamous development of embryos has been reported for *Taraxacum Hieracium Alchemilla Alnus* and *Poa* in which the vegetative embryo sac cell develops from one of the synergids (see Fig 8 7) Embryo development from one of the antipodals has been observed in *Hieracium Elatostema* and *Allium* (Gustafsson, 1946, 1947a, 1947b)

19 1 2 7 Apomixis and Polyploidy Polyploidy is found in many apomicts Many polyploids would not have survived without apomixis Within groups of plants, the diploid species may have entirely sexual behavior while their polyploid relatives are mainly apomicts But there are exceptions In the genera *Allium Agave*, and *Lilium* diploid species with the vegetative form of apomixis, vivipary, do occur But in a large number of genera, the viviparous species are nearly all polyploids Examples are *Polygonum viviparum* (x = 10, 2n = c 88, c 100, c 110, c 132, Darlington and Wylie, 1955, Flovik, 1940, Löve and Löve, 1948, Skalinska, 1950), *Ranunculus ficara* (x = 7, 2n = 32, c 40 Bocher, 1938, Maude, 1939), *Cardamine bulbifera* (Stebbins, 1950), *Saxifraga* spp , various species of *Festuca, Poa Deschampsia*, and other Gramineae
Among the many groups of gametophytic apomicts, the polyploids far outnumber the diploids Examples are *Potentilla, Hieracium* and *Ranunculus* (Stebbins, 1950) Other groups are exclusively polyploid Stebbins (1941) lists 24 gametophytic apomicts that are polyploids Later, he mentions four additional ones, *Parthenium Rudbeckia Paspalum*, and *Crataegus* (Stebbins, 1950)

19.2 Parthenogenesis in Animals

In higher animals apomixis occurs almost exclusively as parthenogenesis There are two kinds of parthenogenesis in animals **haploid parthenogenesis and diploid parthenogenesis.**

Haploid parthenogenesis often occurs in the form of male haploid genetic systems in which the males arise by parthenogenesis from unfertilized eggs **Male haploidy** is restricted to only a few higher taxa (Hartl and Brown, 1970) It occurs in some insect families of the orders Hymenoptera, Homoptera, Coleoptera, and Thysanoptera It also occurs in the aquatic Rotifera and the Acarina mites In the Hymenoptera for instance, the haploid eggs parthenogenetically develop into males while the fertilized eggs develop into females Spermatogenesis in the haploid males occurs without chromosome reduction Usually only one equational meiotic division occurs Haploid parthenogenesis is almost exclusively **facultative parthenogenesis,** since an egg may either be fertilized or develop parthenogenetically In diploid parthenogenesis, only diploid females are produced from unfertilized eggs They are genetically identical to their mothers Two types of diploid parthenogenesis exist **obligatory parthenogenesis** and **cyclical parthenogenesis.** In obligatory parthenogenesis, this is the only form of reproduction The population is entirely made up of females If an occasional male is found, its presence is not a prerequisite for species survival Obligatory parthenogenesis in animals is often associated with polyploidy

In cyclical parthenogenesis, diploid parthenogenesis alternates with sexual reproduction Cyclical parthenogenesis has been demonstrated in the Trematoda, Rotifera, Cladocera, aphids, Diptera, Coleoptera, and Hymenoptera

A classic example for cyclical parthenogenesis is the aphid *Tetraneura ulmi* (White, 1973) Fertilized eggs of this aphid overwinter in Europe, and in spring each egg develops by viviparous parthenogenesis into a small female nymph that forms a gall on the leaves of the primary winter host plant, the European elm Inside the galls, the nymphs develop into adult wingless female aphids called **fundatrices** (Fig 19 4) Each fundatrix parthenogenetically produces about 40 winged daughters called **emigrantes.** These make their way out of the galls and migrate to the roots of the secondary summer host food plants, which are various species of grasses While on these plants, the female emigrantes parthenogenetically produce several generations of female **exules.** The last generation of exules includes winged sexual males and parthenogenetic females (**sexuparae**). These fly back to the primary winter host, the elm, where they parthenogenetically produce male and female **sexuales.** These then pair and produce the fertilized eggs that overwinter on the elm

The female fundatrices, emigrantes, exules, sexuparae, and sexuales of *T ulmi* all have $2n = 14$ But the male sexuales have $2n = 13$ (Schwartz, 1932) This constitutes an XO XX sex-determining mechanism since the females have 2X and the males 1X At the end of the warm season, the sexuparae parthenogenetically produce two kinds of eggs that will develop into female and male sexuales From one kind of egg, a female with a normal $2n = 14$ number of chromosomes develops by a single maturation division that will produce $n = 6 + X$ eggs The details of this ameiotic parthenogenetic egg maturation process had been unresolved for a long time They have been elucidated by Cognetti (1961a, 1961b, 1961c, 1962) and Pagliai (1961, 1962) for *Brevicoryne brassicae, Macrosiphum rosae, Myzodes persicae,* and *Toxoptera aurantiae* Synapsis and bivalent pairing occur normally However, the bivalents then separate again into univalents without spindle for-

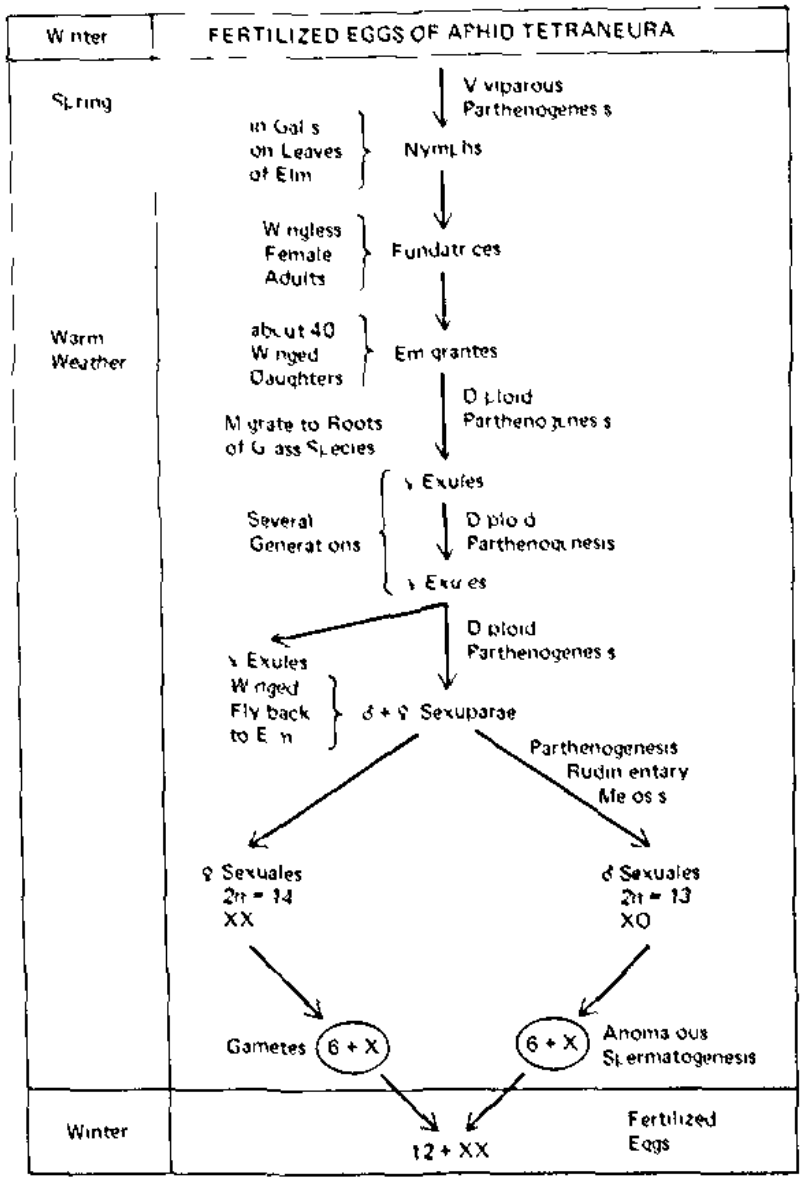


Fig. 19.4 Cyclical parthenogenesis in *Tetraneura ulmi* (After White, 1973)

tion. Subsequently, a single mitotic maturation division occurs with the diploid chromosome set, and the formation of a diploid polar body aids in the elimination of the extra set of chromosomes. The polar kinesis has been described fairly early (Blochman, 1887, Stevens, 1905, Tannreuther, 1907). The other kind of egg develops by a **rudimentary meiosis.** Only the two X chromosomes pair and segregate meiotically. One of the two X chromosomes goes to one cell pole and remains single. At the other pole, all 12 autosomes and the other X chromosome come together and form the XO eggs that develop into male sexuales.

The spermatogenesis of the male sexuales is very anomalous. The autosomes pair and form a first meiotic metaphase plate with the X remaining univalent. During

embryos show microcephaly (abnormal smallness of head) lordosis (abnormally exaggerated forward curvature of the spine), and ventral odema (puffy swelling of the belly) After hatching the animals are sluggish inactive and with abnormal muscle tissue The animals have a high death rate and almost all affected animals die at an early stage

Adult parthenogenesis is known in fishes amphibians reptiles and birds Parthenogenetic embryos occur in mammals Mice were inseminated with x rayed and ultraviolet light treated spermatozoa and embryos were examined in the blastocyst stage (Edwards 1954 1957a 1957b) Nitrogen mustard toluidine blue and trypaflavine also were applied (Edwards 1954 1958) At certain blastocyst mitoses at higher x ray dosages *normal chromosomes were observed in haploid number* presumably the maternal ones and in addition abnormal or fragmented ones presumably the paternal ones Development of the embryos was retarded Similar experiments were carried out in rat hamster guinea pig rabbit sheep and ferret (Beatty 1967) Pincus (1939a 1939b) claimed that he treated rabbit virgin eggs with hypotonic salts, transferred them into host females and that two female young were born Thirty years have passed since these first and only reports of the survival of such rabbit parthenogenones to birth but no confirmation has been reported since

Part IX
Extrachromosomal Inheritance

Parts II through VIII of this book dealt with the chromosomes as the hereditary determinants. However, it was recognized fairly early that the chromosomes are not the only carriers of genetic factors. Throughout the history of genetics, reports seemed to indicate that extranuclear elements could be possible agents of hereditary transmission. Wettstein in 1928 coined the expression plasmon with which he wanted to signify the cytoplasm as a hereditary agent. Part IX deals with such extrachromosomal genetic factors.

Chapter 20
Plastids, Mitochondria, Intracellular Symbionts, and Plasmids

A **plasmagene** (Darlington, 1939b) can be defined as an extranuclear hereditary determinant that shows non Mendelian inheritance Goldschmidt (1945) proposed that the term should be used only in such instances where a self-replicating unit in the cytoplasm produces definite genetic effects similar to those produced by genes in the chromosomes The sum total of all plasmagenes constitutes the plasmon or the **plasmotype** (Imai, 1936) The plasmotype and the genotype are referred to as the **idiotype** (Siemens, 1921) or the entire genetic system of the cell At the time the concept of the plasmon was established, the details of extrachromosomal inheritance were not too well known Now it seems to be clear that most of the cases of cytoplasmic inheritance could be included in one of the following three groups

1 plastids and mitochondria
2 intracellular symbionts
3 plasmids

20.1 Plastids

The first evidence of cytoplasmic inheritance came from studies that involved plastid characteristics In general, reciprocal crosses had shown the equality of genetic contributions from both parents This was already shown by Kölreuter (1761–1766) (Chapter 1) But if cytoplasmic hereditary determinants are taken into account, the contribution from the egg is much greater than that from the sperm Plastid characteristics are therefore inherited from the female parent The discovery of such plastid inheritance was presented by Correns (1902, 1909) He studied the four-o'clock plant *Mirabilis jalapa* The leaves of this plant have normal dark green chloroplasts but in the *albomaculatus* type, there are variegated leaf areas that have chlorophyll-deficient chloroplasts that cause pale green, pale yellowish, or white patches Flowers on entirely dark green branches produce seeds that grow into normal dark green plants Seeds that develop on variegated branches produce 3 kinds of progeny green, variegated in variable proportions, and white Seeds from entirely white branches produce progeny entirely deficient in chlorophyll The pollen source is of no influence to the development of the progeny Consequently, inheritance is entirely maternal and determined by cytoplasmic factors

The evidence that hereditary material is included in the chloroplasts was finally shown in electron microscopic studies In 1962 Ris and Plaut demonstrated by electron microscopic and cytochemical methods that chloroplasts in the alga *Chlamydomonas moewusii* actually contain DNA (Chapter 1) Chloroplasts are reported to have as much DNA as bacteria (Ellis 1969) Reports of DNA in the alga *Acetabularia* are 10^{-5} grams per plastid (Green and Burton, 1970) and 10^{-14} grams per plastid in the algae *Chlorella euglena* and *Chlamydomonas* (Brown 1972) The total DNA content of a chloroplast is usually about 10 times that of a mitochondrion (Granick and Gibor 1967) The extranuclear hereditary determinants of chloroplasts can be likened to the genophores of bacteria They very much resemble pure DNA and differ from nuclear chromosomes by carrying much less protein and by lacking histone in their structural organization Plastid DNA as bacterial DNA is circular in structure (Manning et al 1971 Sprey and Geitz 1973 Ellis, 1974 Falk et al 1974 Herrmann et al 1974)
Replication of chloroplast and mitochondrial genophores was demonstrated by means of multiple **displacement loops** or **D-loops** (Kasamatsu et al 1971 Kolod ner and Tewari 1973) These are short three-stranded closed circular DNA regions, approximately 10 000 base pairs apart They expand undirectionally toward each other and upon meeting appear to initiate the formation of a double stranded replicative fork structure Chloroplast DNA is unique in that it is specifically related to ribosomal and transfer RNA as revealed by DNA-RNA hybridization studies (Tewari and Wildman 1970) Isolated chloroplasts can carry out protein synthesis (Heber, 1962) According to DuPraw (1970) intrachloroplast proteins sometimes account for 70% to 80% of the total leaf proteins But protein synthesis in the chloroplasts is not entirely autonomous as has been demonstrated in studies by Kirk (1966) Normally mutations in the plant nucleus chemically change the enzymes that synthesize chlorphyll Consequently, not all chloroplast proteins are coded by chloroplast DNA Apparently the synthesis of chloroplast structures is carried out by the combined effort of chloroplast genophores and nuclear genes

20.2 Mitochondria

In 1940 Winge and Lautsen demonstrated mitochondrial inheritance of germination in yeast *Saccharomyces* Ephrussi et al (1949) and Ephrussi (1953) induced the so-called *petite* (*p*-) type mutant in the yeast, *S cerevisiae* using the DNA-specific acriflavine dye, tetrazolium, and ultraviolet radiation This caused respiration deficiencies affected by an inability of the mitochondria to synthesize certain respiratory enzymes Slonimski (1949) demonstrated that mitochondrial respiration almost completely ceased, this was confirmed by Yotsuyangi (1962) who also noticed the lack of cytochromes of certain dehydrogenases and membranes It is now well established that these deficiencies are caused by large deletions or even complete loss of mitochondrial DNA Such loss has been genetically demonstrated by the absence of one, several, or all genetic markers in the mitochondrial genophore (Faye et al , 1973, Nagley and Linnane, 1972, Nagley et al ,

1973 Uchida and Suda 1973) The deletions are compensated by repetition of nondeleted genophore segments of mitochondrial DNA (Hollenberg et al., 1972 Van Kreijl et al 1972, Faye et al., 1973)

Additional evidence linking mitochondria to the inheritance of certain characteristics was the discovery of the so-called *poky* type mutants in the fungus *Neurospora crassa* (Mitchell and Mitchell, 1952) This slow growth characteristic, which cannot be supplemented by growth factors is inherited only through the female and shows no transmission patterns through the male

A significant finding similar to that in chloroplasts was the discovery of mitochondrial DNA under the electron microscope (Nass and Nass 1962, 1963) (Chapter 1) Establishment of the fact that mitochondrial DNA like plastid DNA can be circular in shape followed soon (Kroon et al., 1966 Nass 1966 Sinclair and Stevens 1969) But not all mitochondrial DNA is circular as was reported for *Neurospora* (Shapiro et al., 1968) and *Phaseolus* (Kirschner et al., 1968) Restriction enzyme mapping of mitochondrial DNA has been reported for *Neurospora* (Bernard et al., 1975a, 1975b) yeast (Sanders et al., 1975a, 1975b) mouse, monkey and humans (Brown and Vinograd, 1974 Robberson et al., 1974) It is now well established that the mitochondrial DNA molecules of fungi and plants are substantially larger (50-70x10^6 daltons) than those of vertebrates (10x10^6 daltons) (Dujon and Michaelis 1974) That the gene sequence of DNA within the mitochondrion is completely different from that in the nuclear genome of the same species has been established by Tabak et al (1973) and Flavell and Trampe (1973)

20 3 Intracellular Symbionts

Symbiotic organisms are those that have established such an intimate relationship with their host cells that they behave as if they were cellular inclusions Those symbionts are subject to hereditary transmission and it is difficult to distinguish whether they are subject to heredity or infection.

20 3 1 The P Particles of *Paramecium*

This is a group of some 10 different Gram-negative bacterial symbionts including *kappa gamma delta pi mu lambda alpha* and *tau* that occur in the cytoplasm of *Paramecium aurelia* and are called collectively P particles (Sonneborn, 1959) They were first reported by Sonneborn in 1938 They are comparatively large particles that consist of DNA, RNA, protein, and related substances The first demonstration of their effect was presented by Sonneborn in 1943 when he showed that cytoplasmic *kappa* particles caused a killer trait in *Paramecium*. But the killer trait was also dependent on the genotype of the *Paramecium* cell. Reproduction of *kappa* particles occurs only in cells containing the genes *K*, s_1 and s_2. Cells with the genotype *Kk* contain only half the number of *kappa* particles that are contained in cells with genotype *KK*. About 400 particles per cell were postulated to be required for the killer effect. When Preer (1950) succeeded in stain

ing the particles by the DNA specific Feulgen method the expected number of *kappa* particles was observed microscopically Some races of *Paramecium* called **killers,** produced substances (paramecin) that had a lethal effect on members of other races, called **sensitives.** A medium that contains killers for a time and is then replaced by sensitives causes the sensitives to be killed A certain killer strain say *kappa* is protected against the killer activity of its own homologous *kappa* particles, but it is sensitive to killer particles of other types like *pi* or *mu*
The killer particles have different modes of killing sensitives There are 6 different killer types (Siegel 1953, Sonneborn, 1959)

1 *Vacuolizer* The killer eliminates the sensitive by vacuolizing it
2 *Humper* The killer causes the sensitive to form humps
3 *Spinner* The killer makes the sensitive rotate
4 *Paralyzer* The killer paralyzes the sensitive
5 *Rapid Lysis* The killer eliminates the sensitives very quickly
6 *Mate Killer* Killing of sensitives occurs only during conjugation

Work with symbionts in paramecium has been reported by Preer et al (1972) Franklin (1973), Gibson (1973), Karakashian and Karakashian (1973) and Soldo and Godoy (1973) *Lambda* killer particles seem to contain multiple copies of each DNA sequence This is in contrast to most free-living bacteria which have only one or a few copies of a given DNA sequence Soldo and Godoy speculated that this may be a consequence of adaptation resulting from prolonged intracellular existence *Mu* particles could be removed from *Paramecium* with penicillin which seems to prove that some killer particle cell walls are similar to those of certain bacteria (Stevenson, 1965, Franklin, 1973) *Kappa* particles, however, were unaffected by penicillin (Williamson et al, 1952) It has been discovered that a certain percentage of every *kappa* particle population contains so-called proteinaceous **R-bodies,** which are refractile inclusions consisting of thin ribbons of protein that are wound into a tight roll of 10 to 12 turns R-bodies are responsible and essential for the toxic action of *kappa* particles on sensitive paramecia After sensitives ingest *kappas*, the *kappas* begin to break down in their food vacuoles The freed R-bodies then suddenly unroll or unwind into a long twisted ribbon 15μm long, 0 2-0 5μm wide, and 12 nm thick The food vacuole membrane breaks down and the killing process is initiated (Jurand et al, 1971) The remarkable structure and behavior of the R-bodies is unparalleled with any other bacterial structure known According to Preer et al (1966), no other bacterial structure is able to undergo such extensive and reversible changes in form The bacterial nature of *kappa* particles has been established by their electron microscopic structure and chemical composition (Dippell, 1959, Smith Sonneborn and Van Wagtendonk, 1964, Kung, 1971)

20 3 2 The Sigma Virus in *Drosophila*

This virus was discovered in *Drosophila melanogaster* in 1937 by L'Heritier and Teissier It makes its host CO_2-sensitive *Drosophila* can be easily anesthetized with CO_2 and usually recover fast and completely when the CO_2 is removed But certain *Drosophila* strains were discovered that become permanently paralyzed by CO_2 exposure Such strains occur naturally in different countries of the world

(Kalmus et al., 1954) Sensitive strains of *Drosophila* after brief CO_2 exposure become "drunk" or uncoordinated and some of the legs become paralyzed Reciprocal crosses have been carried out that demonstrated that inheritance was mainly maternal Repeated backcrossing of sensitive females to normal resistant males yielded sensitive offspring only Backcrossing of normal females to sensitive males produced a few sensitive progeny, but mostly the trait was not passed on
The virus can be injected in the form of extracts into normal resistant flies in order to induce CO_2 sensitivity Such injection infected females do not regularly transmit their sensitivity to the offspring, injected males never do Strains infected by injection are called **nonstabilized lines.** In the original **stabilized lines** the virus is believed to be located intracellularly in the germ line in a noninfectious form, maybe as naked nucleic acid, and is transmitted hereditarily (L'Héritier, 1962, Seecof, 1968) Infective virus could be produced by the maturation of the noninfectious form *Drosophila* has nuclear genetic resistance to the *sigma* virus Flies homozygous for a resistance factor *re* do not become infected after inoculation with *sigma* virus (Gay and Ozolins, 1968)
The *sigma* virus could not be isolated (Plus, 1962, Seecof, 1962), but electron microscopy has demonstrated it in *sigma*-bearing lines (Berkaloff et al 1965) The virus particles are rod-shaped, 7 nm by 140 nm in size Plus (1963) suspected that *sigma* is a DNA virus

20 3 3 The Maternal Sex-Ratio Condition in *Drosophila*

The progeny of some *Drosophila* strains is entirely female The original accounts of this trait were governed by nuclear genes as reported earlier (Section 18 1) But instances of maternally inherited sex ratios (*SR*) conditions are also known Such conditions were reported for several species of *Drosophila* specifically *D bifasciata D prosaltans, D willistoni D paulistorum D equinoxialis D nebulosa* and *D robusta* (Magni, 1954, Cavalcanti et al., 1958, Malogolowkin, 1958, 1959, Oishi and Poulson, 1970) Death of males was found mainly in early embryonic stages but usually as zygotes Maternal sex ratio condition is either revealing total absence of males in the progeny, as in *D willistoni*, or predominantly female with a few male offspring, as in *D paulistorum*
The sex ratio condition in *D willistoni, D equinoxialis, D nebulosa*, and *D paulistorum has been linked to the occurrence of treponema-like SR spirochaetes* that are 5μm to 6μm long and 0 1-0 2μm wide in their filamentous stage and exhibit a typical spiral form (Malogolowkin, 1958, Malogolowkin et al., 1960, Poulson and Sakaguchi, 1960, Oishi and Poulson, 1970) If SR spirochaetes of any of these *Drosophila* species are mixed either *in vitro* or *in vivo*, spirochaetes of one or of both species die (Sakaguchi and Oishi, 1965, Sakaguchi et al., 1965, Oishi and Poulson, 1970) Oishi and Poulson have demonstrated that the cause of death of these spirochaetes is a DNA containing spherical virus of 50nm to 60nm in diameter Preer (1971) suggested that the same virus may be the agent that kills developing male *Drosophila* and causes the development of entirely female strains In the species *D bifasciata* no spirochaete bacteria are present, and a virus is suspected of killing the male zygotes instead (Ikeda, 1965, Leventhal, 1968) Another *Drosophila* species with sex ratio condition not caused by spiro-

chaetes is *D robusta* (Poulson 1968) The cause of male death in *D prosaltans* has not been completely resolved (Poulson, 1963) The SR spirochaetes are believed to be mycoplasma-related (Williamson, et al , 1977)
Mycoplasmas are the simplest known cellular organisms Their size overlaps with the largest viruses and the smallest bacteria They have a circular DNA molecule that is not separated from the remainder of the cell Unlike viruses they do not require host cells for duplication Their plasma membrane is not surrounded by an elaborate cell wall as in bacteria (Novikoff and Holtzman 1976)

20 3 4 The Milk Factor in the Mouse (MTV)

Bittner (1938, 1939) is credited with discovering an extranuclear factor responsible for the susceptibility of mice to mammary cancer The factor was shown to be transmitted through the milk It was believed to be related to the viruses being a particulate nucleoprotein (Barnum et al 1944) It is now known as the mouse mammary tumor virus (MTV) Moore (1967) and Hageman et al (1968) established that the virion of MTV is the B particle which was described by Bernhard (1958) The presence of MTV has been demonstrated in the gametes of mice by electron microscopy, immunofluorescence, and bioassay (Bentvelzen et al , 1970) This form of cancer was detected by outcrossing strains of mice that were inbred *for many generations About 90% of the mice of these inbred strains over* 18 months of age had breast cancer When females of these strains were crossed with inbred strains that had low incidence of cancer, 90% of the F_1 individuals had breast cancer If the reciprocal cross was carried out, none of the F_1 had breast tumors Mice of a cancerous line fed from birth by noncancerous foster mothers did not show evidence of tumors But after injection with blood from cancerous mice, they did develop tumors (Woolley et al , 1943) Bentvelzen et al concluded that host genes control the susceptibility to MTV

20 3 5 Cytoplasmic Male Sterility (CMS)

CMS has been reported for 80 species, 25 genera, and six families (Edwardson, 1970) Viruses can be transmitted by the seed and induce pollen sterility This has been demonstrated with the tobacco ringspot virus (TRSV) in *Petunia* (Henderson, 1931) and with the tomato ringspot virus (TMSV) in soybeans (Kahn, 1956) Atanasoff (1964a, 1964b) suggested that all cytoplasmically inherited traits such as cytoplasmic male sterility (CMS) could be due to viral infections even though *the presence of a virus has not been demonstrated The successful* asexual transmission of CMS through plant grafts demonstrated in *Petunia* (Frankel, 1956, 1962, 1971, Edwardson and Corbett, 1961, Bianchi, 1962) and in sugar beets (Curtis, 1967) supports the possible assumption that CMS could be transmitted by virus CMS has been demonstrated in many plant species A plausible definition for cytoplasmic male sterility is a condition in which pollen sterility is at least partially caused by factors that are only passed on by the female and in which this pollen sterility is not abandoned during successive reproductive generations The expression of this condition can also be influenced by chromosomal genes The expression of cytoplasmic male sterility can often be changed by the condi-

tions of the specific environment in which the plants are grown The present usability or the potential use of CMS for the production of hybrid seed has been demonstrated in tobacco (Chaplin 1964), maize (Rogers and Edwardson, 1952), sorghum (Ross, 1971), and wheat (Wilson and Ross, 1962) Other reports of economic possibilities with CMS are for flax (Chittenden, 1927), onion (Jones and Clarke 1943), alfalfa (Davis and Greenblatt, 1967), petunia (Edwardson and Warmke, 1967) sugar beets (Theurer and Ryser, 1969), intermediate wheatgrass (Schulz Schaeffer, 1970), cotton (Meyer, 1971), and other crops

The phenomenon of cytoplasmic male sterility was first described by Correns in 1904 and was interpreted as a true case of CMS by Wettstein in 1924 There are 4 possible sources of CMS

1 intergeneric hybridization and substitution backcrossing
2 interspecific hybridization and substitution backcrossing
3 intraspecific hybridization
4 spontaneous occurrence

A good example for the development of cytoplasmic male sterility through intergeneric hybridization is wheat Kihara (*1951a*) crossed *Aegilops caudata* with *Triticum aestivum* and backcrossed the hybrid with *T aestivum* Since the female parent contributes the majority of the cytoplasm, the backcrossing accomplished a replacement of the *Aegilops* chromosomes by *Triticum* chromosomes, which were placed in the *Aegilops* cytoplasm CMS was the result of this method

Interspecific hybridization was the approach of obtaining CMS in tobacco when Burk (1960) placed the genome of *Nicotiana tabacum* into the cytoplasm of *N bigelovi* Cytoplasmic male sterility in onions was accomplished by intraspecific hybridization (Jones and Emsweller, 1937, Jones et al , 1939, Jones and Clarke, 1943, Jones and Davis, 1944, Jones, 1946) A recessive gene (*ms*) in the homozygous state caused plants to be male sterile when by appropriate crossing it was placed into a certain type of cytoplasm (*S*) The same gene had no effect in a different type of cytoplasm (*N*)

And finally, the most prominent example for spontaneous occurrence of CMS is maize The Texas source (T type) was isolated from the cultivar "Golden June", a variety of dent maize that was grown in the southwestern United States (Rogers and Edwardson, 1952) Another cytoplasm used in maize breeding is the S type Maize inbred lines that were sterile in the S cytoplasm were not necessarily sterile in the T cytoplasm Because a higher percentage of corn belt inbred lines were completely sterile in the T-cytoplasm than in the S-source, most hybrid production was based on the T cytoplasm (Duvick, 1966) It has been established that two plasmid like DNA's are unequally associated with mitochondrial preparations from S type maize (Pring et al., 1977)

20 4 Plasmids, Episomes, and Transposable Elements

Plasmids (Lederberg, 1952) in the strictest sense are extrachromosomal hereditary determinants that only occur in an autonomous condition and exist, replicate and are transferred independent of the chromosomes This concept excludes the

category of episomes, which in contrast are hereditary determinants that can alternate their autonomous existence with a condition in which they are attached to the chromosomes Interpreted this way an episome that is detached from its chromosome becomes a plasmid (Hayes 1968 Novick 1969 Richmond 1970) The term episomes was coined by Jacob and Wollman and was adopted from *Drosophila* genetics (Thompson 1931) **Insertion sequence elements** (*IS*) and **transposons** are much smaller than plasmids and episomes Three classes of ele ments that are able to insert at different sites of DNA molecules can be distinguished

1 *Conjugons* (Luria 1963) These are specialized genetic promotor elements that are necessary during bacterial conjugation in order to establish contact between cells Cells that possess conjugons (**donor cells**) can establish contact with related cells that lack such episomal conjugons (**recipient cells**).
2 *Temperate bacteriophages* (Jacob et al 1953) These are bacterial viruses that can enter lysogenic bacteria and in contrast to virulent or lytic bacteriophages that destroy their host by the process of lysis they become integrated into the bacterial genophore or become attached to it without damage to the host Phages when integrated by this process are called **prophages** (*Lwoff and Gutman 1950*) Lysogenic bacteria become immune to further infection by an extrinsic homologous phage after integration of a prophage The sites of integration of prophages into the bacterial genophore (prophage sites) are designated on the genetic map by a Greek letter
3 *Transposable genetic elements* These are the IS elements and transposons They are small nonreplicating transposable DNA that can be inserted into the DNA of tem perate bacteriophages or plasmids They have not been found to exist autonomously

According to Rieger et al (1976) conjugons have three functions

1 the determination of the surface properties and synthetic abilities associated with the establishment of effective contacts between conjugation partners
2 mobilization of the genophore material that is to be transferred from the donor cell to the recip ent cell
3 provision of the energy source required for genophore transfer

20 4 1 The F-Episomes

Also called the F agent this conjugon is an episome or a transmissible plasmid At this point it is important that the student understands all these different terms because they are used interchangeably in the literature The F episome is part of a group of sex factors that are capable of inducing conjugation The presence or absence of these factors in a bacterial cell determines its sex

Depending on their organization there are 4 kinds of bacterial cells (Demerec et al 1966)

1 F cell
2 F cell
3 Hfr cell
4 F cell

An F cell is a bacterium that does not posses an F episome Such a bacterium is a genetic recipient cell or conjugation "female that does not possess the ability of a genetic donor cell or male Such a cell cannot transmit an F episome but it can be infected with such an episome (Fig 20 1)

An F cell is a bacterium that carries an F episome extrachromosomally Such

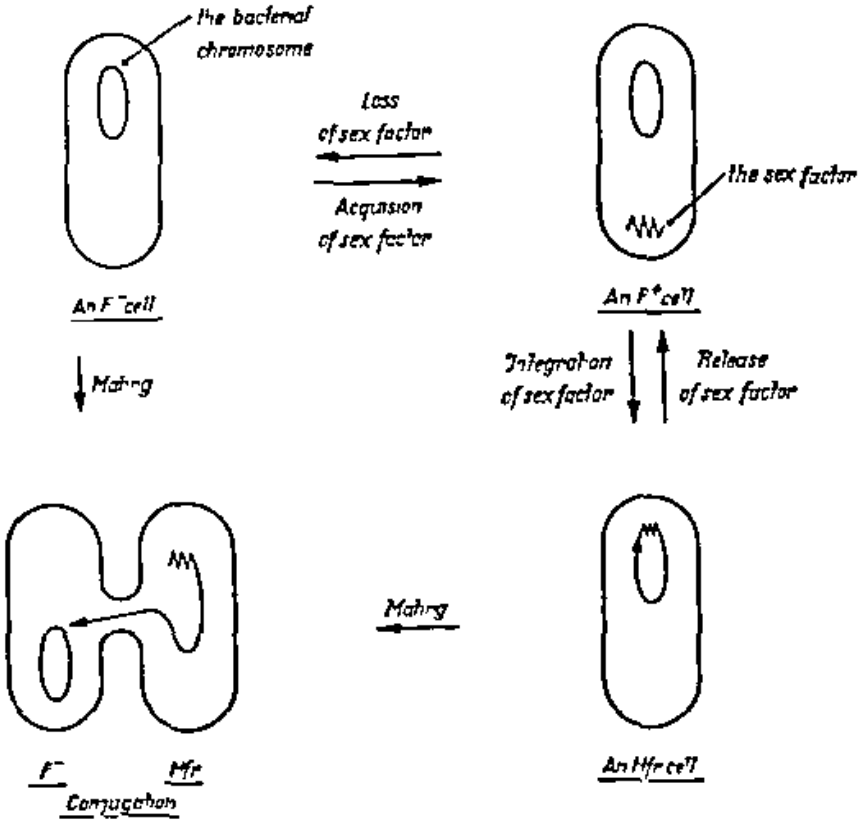


Fig 20 1 Different manifestations of the F episome in *Escherichia coli* (From Scaife, 1963)

episome can be transferred to an F cell with high frequency Such an F^- cell then becomes an F^+ cell

An Hfr cell is a bacterium that has an F-episome integrated in its bacterial genophore (Fig 20 1) An F^+ cell can convert into an Hfr cell by a process of integration (Demerec et al , 1966) The integrated F-episome confers on the Hfr cell the ability of **high frequency recombination** (Hfr) Due to transient coupling between F and the genophore, the frequency of genetic recombination in matings between F^+ and F cells is about 10^{-6}, but in matings between Hfr and F cells, it is as high as 0 01% to 0 5% The process of bacterial conjugation involves direct contact between the donor and the recipient, which is followed by the establishment of a cellular bridge that enables transfer of the entire male genophore, or only a segment, into the recipient cell Transfer usually results only in a **merozygote** (Wollman et al , 1956), which is diploid for only part of the genophore and haploid for the rest of it This process of mating is illustrated in Fig 20 1 The transfer occurs only after breakage of the circular genophore Breakage happens at one of the two insertion points of the F-episome This point is referred to as "*O*" (origin) or head This portion of the genophore is always the first to enter the recipient cell The position of the Hfr-episome is at the end of the transferring linear genophore Since the genophore usually transfers only in part, the integrated F-episome is included only rarely in the transfer *O* always marks the head and Hfr the tail of the transferring genophore But the sequence of the genetic markers of each Hfr-

strain is strain-specific since Hfr can integrate at different sites of the genophore (Hayes, 1964) If the markers of the genophore are designated by the letters of the alphabet then the following sequences would be possible

O A-B-C-D-E-F-
O-B-C-D-E-F-A-
O-D-E-F A-B C-
O F-A B-C D-E-
or other sequences

The integration of the F episome into the *Escherichia coli* genophore is not entirely random Certain sites on the *E coli* map are preferred

An F′ cell is a bacterium that carries an extrachromosomal F-episome attached to a genophore fragment F′ episomes are also called **F-merogenotes** (Clark and Adelberg, 1962) or **F-genotes** (Ramarkrishnan and Adelberg, 1965) An F′ episome carrying a genophore fragment can interact at a specific site on the bacterial genophore An F′ cell is haploid except for a short genophore segment in which it is partially diploid and heterozygotic Such a cell is also called a **heterogenote** (Morse et al , 1956a) F′ episomes arise when excision during release of the F-episome from an Hfr is not exact

20 4 2 Colicinogenic Factors

Fredericq (1953, 1954) discovered extrachromosomal genetic elements in coliform bacteria that produce proteinaceous substances called **colicins** capable of killing sensitive members of other bacterial strains of the same or closely related species He called these extrachromosomal elements **Colicinogenic factors** (Cf) He proved that Cf can be transferred from colicinogenic (col^+) to noncolicinogenic (col^-) strains by cell contact The col-factors are a heterogeneous group of plasmids, and they are able to carry out a wide variety of activities such as colicin production and release, production of colicin immunity, and quiescent and vegetative reproduction Herschman and Helinski (1967) described two quite distinct classes of col factors colicinogenic sex factors and nontransmissible col factors A well studied example of a colicinogenic sex factor is Col V-k 94 It can mobilize the genophore but it is not integrated In addition to a sex factor, it carries a structural gene for a colicin and a gene for resistance or immunity to the colicin Col V k 94 was studied more extensively than any other colicinogenic sex factor According to electron microscopic studies by Bradley (1967), nontransmissible col-factors such as colE1 are defective phages that have the ability to produce incomplete, noninfective phages

20 4 3 Resistance Factors

The resistance or **R-factors** (Iseki and Sakai, 1953) of the coliform bacteria are capable of conferring resistance to antibiotics or to metal ions (Summers and Silver, 1972) Such factors confer selective advantage to organisms growing in the presence of antibiotics or having metal complexes such as mercurials and have been isolated from hospital environments (Joly and Cluzel, 1975) There are two

classes of resistance factors nontransmissible R-factors and transmissible RTF sex factors

Nontransmissible R-factors consist of DNA that provides resistance to one or several antibacterial drugs or antibiotics such as chloramphenicol, sulphonamide, tetracycline or streptomycin and/or several metal ions such as *Hg*, *As* and *Cd* For instance in studies of *Shigella* bacteria it was found that strains resistant to all four of these commonly used drugs were more common than strains resistant to only one two or three **Multiple drug resistance** is now an established fact Resistance to kanamycin, neomycin, and the penicillins is very often included (Lewin 1977)

In order for an R-factor carrying bacterium to be able to conjugate with other bacterial strains and to transfer drug resistance to them, the R-factor has to be linked to a **resistance transfer factor** (RTF) (Watanabe and Fukasawa, 1961) RTF often carries resistance to ampicillin only Such an R-RTF complex is very similar to the F-episome (Section 20 4 1) but its transmission frequency from donor to recipient is not as high as that of the F-episome The functional relationship between R-RTF complexes and F-episomes is also demonstrated by Watanabe's (1963) discovery that one class of R-factors, fi^+ (**=fertility inhibition**), inhibits the genetic expression of the F-episome when it is acquired by the F^+ recipient cell Inhibition of the F-episome expression caused by an fi^+ R factor seems to be caused by a single gene in the R-factor (Hirota et al , 1964)

The presence of antibiotics in livestock feed and as therapeutic agents as well as the use of heavy metals such as mercury as disinfectants has contributed to selection of R factors, and transmission to human or animal pathogens leads to the difficulty in treating subsequent infections (Meyers et al , 1975)

20 4 4 Bacteriophages

In Section 20 4, two classes of episomes were described, conjugons and temperate bacteriophages One of the best known is the small bacteriophage *lambda* It is composed of a protein coat containing a single chromosome that consists of a single double stranded DNA molecule This chromosome can be either linear or circular Genetically, this chromosome acts as if it were linear having a definite head and a definite tail The very ends of lambda DNA are single stranded and complementary to each other in nucleotide sequence They are referred to as **sticky ends** or **cohesive ends** (Ris and Chandler, 1963, Hershey et al , 1963) and are from 10 to 20 nucleotides in length They can pair and form a closed circular structure (Fig 20 2) Apparently, there is a small amount of protein associated with the lambda DNA The phage lambda is about 17 μm x 2nm in size When lambda is in its integrated state in the bacterial host cell, it usually occupies a unique position within the bacterial genophore (Fig 20 3) It has been demonstrated that other temperate phages also have their own specific attachment sites on the bacterial genophore (Jacob and Wollman, 1957) As part of the bacterial genophore, prophage genes function in precisely the same manner as bacterial genes When lysogenic bacteriophages become excised (released) from the bacterial genophore, they can enter a lytic cycle (Wollman, 1953) Such a cycle can

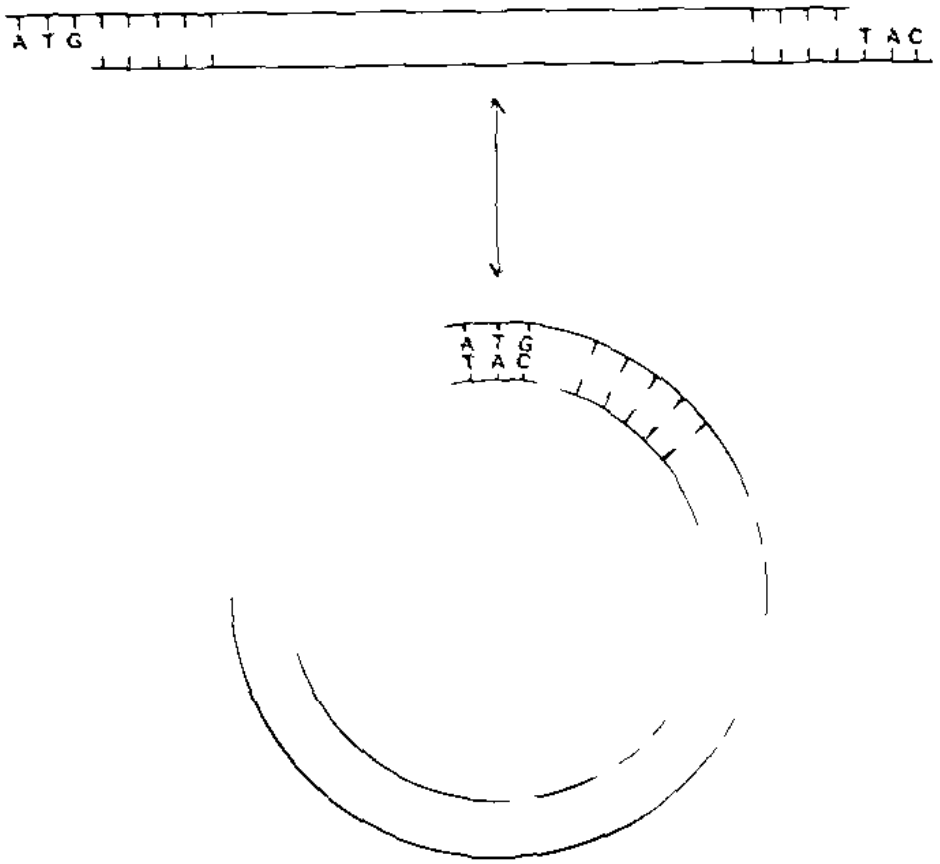


Fig. 20.2. Diagrammatic illustration of the lambda bacteriophage. (After Dr. C. A. Thomas, Jr. From Swanson et al., 1967. Redrawn by permission of Prentice-Hall Inc., Englewood Cliffs, New Jersey.)

be induced, for instance, by ultraviolet irradiation. Inexact excision of phages leads to a linkage of one or more bacterial genes to the phage and formation of specialized transducing phages. In transduction (Zinder and Lederberg, 1952), the phage transfers genetic material from a donor cell to a recipient cell. Since lambda is located close to the galactose genes (see *gal*, Fig. 10.1) of *E. coli*, these are some of the few genes that lambda usually transduces (Morse et al., 1956a, 1956b). The genetic material that can be transduced in such a manner is generally less than 1% of the total length of the bacterial genophore. If lambda picks up a *gal* segment from its previous host cell and transfers it to a new bacterium, the second host cell becomes diploid for the *gal* segment and becomes a **heterogenote.** Usually the phage leaves behind part of itself in the genophore of the first host cell and becomes defective. It cannot become released from the genophore and reproduce. But a defective lambda can be excised again if the new host cell contains another intact lambda helper phage. Transducing lambdas have been investigated physically and genetically. They have lower density, and large middle segments of the genetic map are missing (Arber, 1958; Weigle et al., 1959). Mapping of the lambda bacteriophage has been fairly extensive. According to Lewin (1977)

it seems unlikely that any essential genes have not yet been identified Campbell (1971) summarized genetic data obtained from several laboratories and presented a lambda gene map (Fig 20 4) According to this map lambda has 26 essential and 9 nonessential genes Other temperate bacteriophages that have been described are ϕ80, P1, P2, and Mu

20 4 5 IS-Elements and Transposons

IS-elements are small, transposable DNA segments of 800 to 1400 base pairs This compares with about 46,500 base pairs in the lambda bacteriophage IS-elements can be inserted into bacterial genophores, temperate bacteriophages, or plasmids IS-elements were first detected in the *E coli galT* gene by Jordan et al in 1968 They compared the density of mutated and wild type λ*gal* phages and could show that the mutated phages had increased density After reversion to the wild-type, the density decreased to its usual value Five of such IS-elements are now known in *E coli* others are known in *Salmonella* and *Citrobacter* (Starlinger, 1977) IS-elements can cause the effect of a special kind of mutation, called

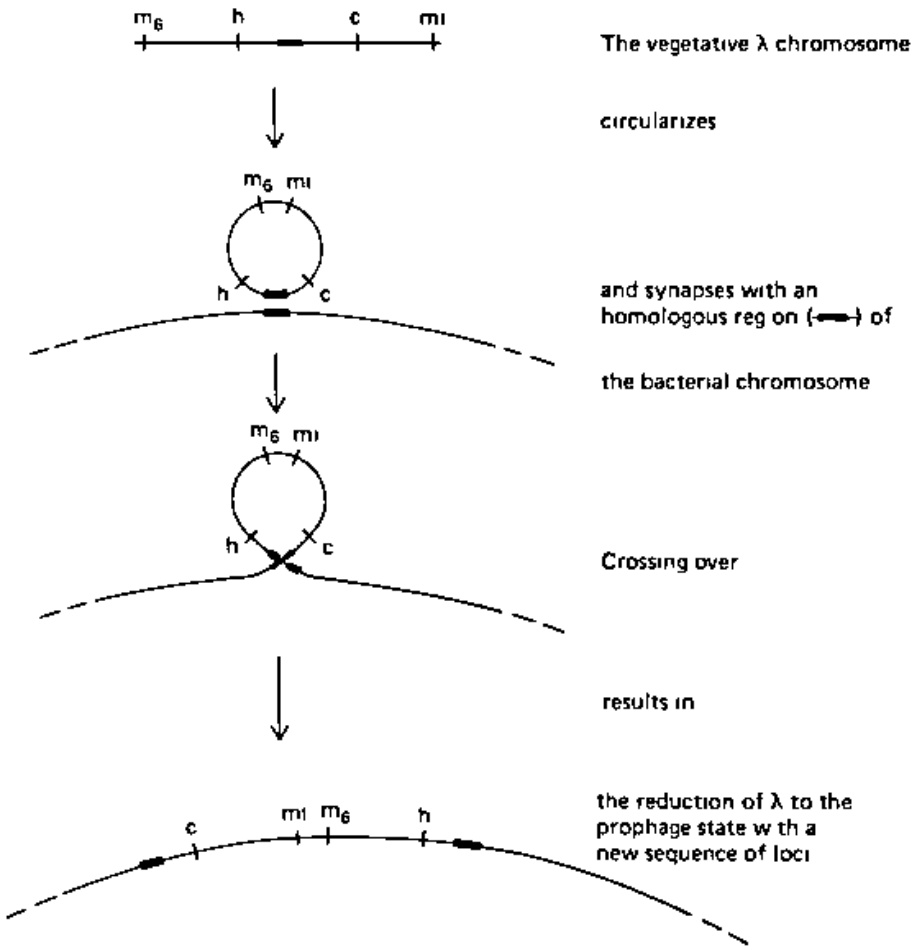


Fig 20 3 Suggested mechanism for the integration of lambda into the bacterial genophore (From Franklin W Stahl, The Mechanics of Inheritance, 2nd Ed , © 1969, p 158 Reprinted by permission of Prentice-Hall, Inc , Englewood Cliffs, New Jersey)

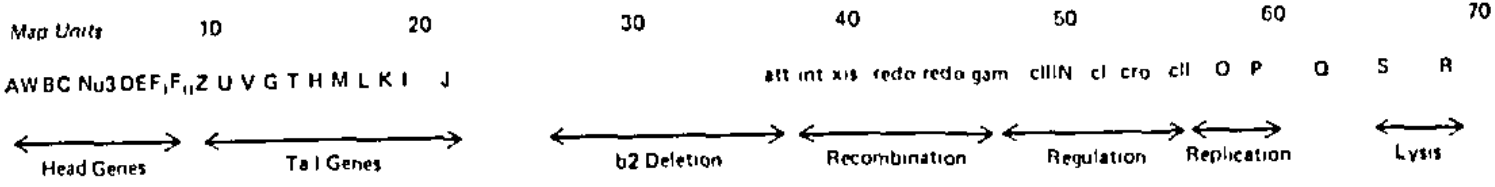


Fig 20 4 Lambda gene map *Genes fall into groups with respective functions* indicated Q-regulator gene (From Lewin, 1977 Redrawn by permission of John Wiley and Sons, New York)

polarity mutation (Franklin and Luria, 1961, Jacob and Monod, 1961b) A polarity mutation abolishes the function of the mutated gene and impairs the function of other genes within the same operon But the polarity mutation caused by the insertion of an IS-element differs from a point polarity mutation in that it easily reverts to the wild-type condition IS-elements have also been demonstrated under the electron microscope (Hu et al , 1975a, 1975b, Ptashne and Cohen, 1975, Saedler and Heiss, 1973, Saedler et al , 1975, Mosharrafa et al , 1976) They were shown by the **heteroduplex technique** originally developed by Davis and Davidson (1968) and Westmoreland et al (1969) The strands of DNA molecules were separated and so-called **heteroduplex DNA molecules** were prepared by DNA-DNA hybridization (Hall and Spiegelman, 1961, Chapter 1) Heteroduplex DNA molecules are hybrid DNA double strands that consist of polynucleotide chains that originated from two different parental molecules The presence of an IS-element in one of the two DNA strands can be seen under the electron microscope as a single strand loop

IS-elements apparently do not exist in the form of autonomously replicating plasmids (Christiansen et al , 1973) Integration of IS-elements shows some site specificity that is intermediate between the strong specificity of lambda bacteriophage and the lack of it in Mu bacteriophage Different IS-elements show different site specificity IS *4* has been observed only at a single site in gene *galT* of *E coli* (Shapiro and Adhya 1969, Fiandt et al , 1972, Shimada et al , 1973, Pfeifer et al , 1977) IS *1* and IS *2* are less specific than IS *4* Several other IS *4* sites have been reported Integration sites of IS *2* and IS *3* in the F-plasmid have been mapped by Davidson et al (1975) and by Hu et al (1975a, 1975b, 1975c) (Fig 20 5) IS-elements have been shown to be partially homologous to the inverted

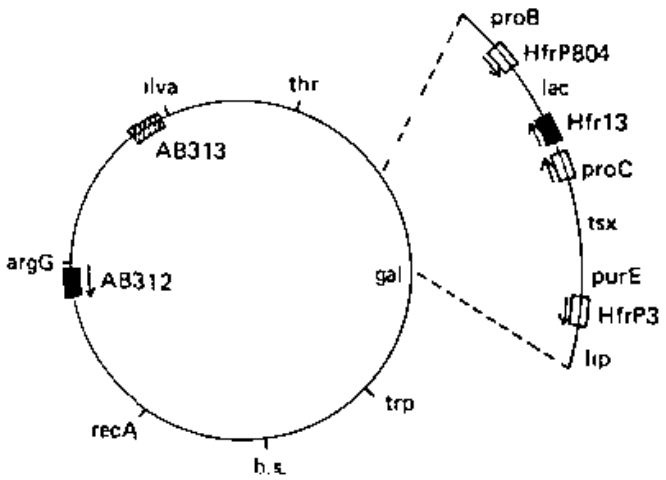


Fig 20 5 Location of some of the multiple copies of IS elements in the chromosome of *E coli* ■ represents IS2, ▭ represents IS3, ▨ is $\gamma\,\delta$ Arrows indicate the orientation of the IS elements and the *Hfr* formed at these positions The integration site of AB313 is taken from Ohtsubo et al (1974) and the integration site of AB312 has been analyzed by Guyer (cited in Davidson et al , 1975) At the position of *Hfr P804* seven other *Hfr* strains are known, at position of *Hfr 13* and the neighboring IS3, six other *Hfrs* and at P3 three other *Hfrs* have been described (From Davidson, Deonier, Hu and Ohtsubo, 1975 Redrawn by permission of the American Society of Microbiology, Washington, D C)

sequences that border the transposon for tetracycline resistance (Cohen and Kopecko 1976) IS *1* an element of 770 base pairs has been analyzed by the nucleotide sequencing technique (Calos et al 1978 Grindley 1978)

Transposons (Hedges and Jacob 1974) are very small DNA segments (100 to 1500 nucleotide pairs) consisting of one or several genes They are capable of insertion and excision in DNA molecules without the requirement of a functional bacterial recombination system They are responsible for resistance to antibiotics They can be transposed from chromosome to chromosome in the same cell (Ptashne and Cohen 1975 Berg et al 1975 Berg 1977a 1977b) The nomenclature of transposons has been explained by Campbell et al (1977) An example would be *Tn 9(Cm)* in which *Tn* is followed by the isolation number and the antibiotic (in parenthesis) to which the resistance is conferred in this instance chloramphenicol Gene mutation occurs through integration of transposons into a gene (Kleckner et al 1975 Berg 1977a) Reversion into wild type generally occurs like that reported for IS elements Inexact excision of transposons can lead to deletions next to the transposon site (Foster 1976 Campbell et al 1977 Botstein and Kleckner 1977 Brevet et al 1977) Also duplications and inversions have been described occurring next to tetracycline transposon *Tn 10* (Botstein and Kleckner 1977) Transposons also show different site specificities (Starlinger 1977)

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