GENETICS *and* METABOLISM

ROBERT P. WAGNER
Department of Zoology
The University of Texas

HERSCHEL K. MITCHELL
Division of Biology
California Institute of Technology

1955

Preface

The purpose of this book is to bring together a variety of facts and ideas derived from the fields of genetics and biochemistry, and, to a lesser extent, from those of physiology, cytology, and embryology, in an attempt to synthesize a general picture of the biochemical basis of inheritance. It is our thesis that inheritance is characterizable in terms of transmission of control of relative rates of biochemical reactions within complex and interlocked metabolic patterns. This is not a new idea, since it has been anticipated in the thinking and by the experimental results of a number of investigators. But even now, in spite of the compilation of a great deal of pertinent experimental data, the ambitious objective of providing complete factual support for the above thesis has not been achieved in this book, as should be readily appreciated from the fact that the title is "Genetics *and* Metabolism" rather than a more pretentious one which would at least indicate that the subject matter discussed is well enough integrated to be described by a single word. In our opinion, such a complete integration has not been attained, nor is it attainable at the present time with present data. It can be shown that a variety of fascinating apparent relationships exist, but more facts are needed to demonstrate either that they are significant or that we have been misled by false clues. Nevertheless, we believe that progress has been such that the time is ripe to present, within the confines of a single volume, a collection of data that have a direct bearing on the problem.

This book has been written primarily for advanced undergraduate or beginning graduate students of genetics, biochemistry, or microbiology. A background of elementary biology, genetics, and biochemistry is assumed. However, in order to make the contents of the book more accessible to more individuals, a short discussion of elementary genetics is given in the first chapter, and some elementary facts of biochemistry and general biology are included where they seem most pertinent.

Although many of the facts discussed are those which may also receive attention in courses in genetics and biochemistry, they are

not, in general, those that occupy central positions of emphasis in formal courses in either subject. Nor are they usually presented in juxtaposition so that the relationships between them become obvious. It is for these reasons that we feel that the material contained herein is not repetitious of that covered in other textbooks.

Material encompassing the general principles included here, with supplements from current advances in the field, have been presented to senior and graduate students in biology and biochemistry in a course given by one of us at the University of Texas for the past seven years. The response of the students has encouraged us to feel that this material provides a creative supplement to the training of those who intend to make the scientific study of life their life's work.

ROBERT P. WAGNER
HERSCHEL K. MITCHELL

April, 1966

Acknowledgments

We wish to acknowledge with gratitude the encouragement, the constructive criticisms, and the generally helpful attitude presented to us by many of our colleagues during the preparation of this book. In particular, we wish to thank Dr. George W. Beadle, of the California Institute of Technology, who made available facilities of the Kerckhoff Laboratories of Biology for two summer periods so that the two of us could work together in writing the manuscript. We are especially indebted also to Dr. Wilson S. Stone, of the University of Texas, who read the entire manuscript and offered many helpful suggestions, as well as to Mary B. Mitchell and Dr. A. H. Sturtevant, of the California Institute of Technology, and Drs. M. R. Wheeler, B. S. Strauss, and R. M. Welch, of the University of Texas, all of whom read parts of the manuscript and provided valuable criticisms. We are most grateful for all this able assistance, but, quite appropriately, ours is the full responsibility for the contents of this book, including the errors that may have been committed.

The art work for the preparation of a number of the figures was done by Mrs. Grace Hewitt Groce. To her and to Miss Nina Stehr, who assisted in the preparation of the manuscript, we offer our thanks for their excellent work.

Contents

CHAPTER 1

Introduction to the Gene Concept

The discussion in this book is almost totally concerned with how genes act, or the effects of their actions, rather than with how they are inherited. But it is nonetheless necessary to know at the outset something about the meaning of the term gene, what exactly it refers to, and how the existence of genes is inferred from experimental evidence. This requires some understanding of the mechanism of Mendelism, and related phenomena associated with sexual reproduction, for it is from these that the modern concept of the gene is derived.

The gene concept is perhaps best introduced by defining the more important terms which are used to describe it. The original definition of the word gene, as given in 1911 by Johannsen (317), the man who coined it, was brief and simple:

> The gene is nothing but a very applicable little word, easily combined with others, and hence it may be useful as an expression for the "unit factors," "elements" or "allelomorphs" in the gametes, demonstrated by modern Mendelian researches.

Johannsen's word filled a definite need from the very beginning of genetics, because it gave geneticists the elements of a vocabulary by means of which they could conveniently separate the idea of cause, i.e., the inherited determination and capacity to develop certain characteristics, from the effects, the characteristics themselves. From gene the term *genotype* was derived to describe the total complement of genes in the fertilized egg, and hence the total capacity to develop certain characteristics (317). To describe the end result, the appearance of the organism resulting from gene action, the term *phenotype* was introduced.

At the time that Johannsen coined the term gene there was little else certain in genetics beyond the occurrence of Mendelian ratios which were obtained when certain types of individuals were crossed. A gene

was recognized then, as it is now, only when it existed in two forms or alleles (contraction of the obsolescent term allelomorph) recognized as being different by their differential effects on the phenotype. Its possible existence as a physical entity was appreciated by only a few of the geneticists of the time, and Johannsen himself was not among them. Proof of the physical existence of genes came when it was conclusively established by Morgan, Sturtevant, Muller, and Bridges (440) that Mendel's units, called genes by Johannsen, were closely connected with visible nuclear structures known to the nineteenth-century cytologists as chromosomes. It then became possible to think of genes as inherited physical entities which express themselves by controlling or determining the processes of development which culminate in the phenotype. These workers demonstrated not only that genes are located on chromosomes but also that they have a linear arrangement on the chromosomes. With the discovery of the significance of the giant salivary gland chromosomes of *Drosophila* by Painter (468), it became possible to identify regions of chromosomes with specific genes and to prove conclusively that each gene occupies a specific *locus* on its chromosome.

1. The Mechanism of Mitosis

One of the chief requirements for an understanding of genetics is an appreciation of the significance of the basic means of cell reproduction among cellular organisms. These processes, mitosis and meiosis, are discussed below in what is thought to be enough detail to provide the uninitiated reader with a background sufficient to understand the fundamentals of the gene concept.

Mitosis is a type of cell division in which a cell gives rise to two daughter cells that possess the same number and same kind of chromosomes found in the mother cell. The process is diagrammed in Fig. 1. The essential factors in the process are: (1) each chromosome within the nucleus about to divide is duplicated—as far as can be determined by genetic means—exactly; (2) the duplicates separate and go to opposite poles of the dividing cell; (3) the distribution of chromosomal material between the two new daughter cells is generally considered to be exactly equivalent, because of the evident elegant mechanism operating to bring about an equal distribution. This is by no means true for the cytoplasm, which has a more homogeneous appearance than the nucleus and appears not to have any mechanism insuring its equal distribution. This point, concerning the distribution of the cytoplasmic

part of the cell, is not of particular importance in the present discussion, but will receive more attention when its detailed consideration becomes important in Chapter 12 in connection with the discussion of cytoplasmic inheritance.

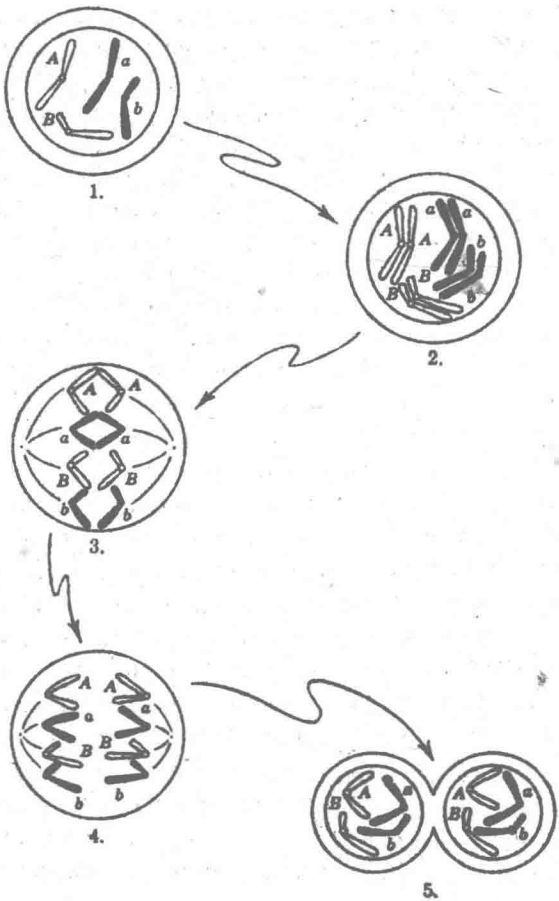


Fig. 1. A diagrammatic representation of mitosis. Some significant stages of mitosis of a cell containing four chromosomes. Stage 1. The chromosomes are visible in the nucleus: early prophase. Stage 2. The chromosomes have been duplicated: late prophase, early metaphase. Stage 3. The duplicates separate: early anaphase. Stage 4. Late anaphase. Stage 5. Telophase and end of mitosis; cytoplasm dividing.

2. The Mechanism of Meiosis

The higher organisms are for the most part diploid ($2N$), which means that they possess two similar sets of chromosomes in each of their cells. This fact has been established in part by cytological and in part by genetical observations. When the gametes of diploid organisms are formed, it can be demonstrated cytologically that the cells which give rise to them undergo a series of two cell divisions which result in a reduction of the chromosome number by one-half, thus producing gametes with a haploid set ($1N$) of chromosomes. The two successive cell divisions resulting in haploidy are together called meiosis. The more specialized terms, *spermatogenesis*, for meiosis producing sperm cells, and *oögenesis*, for that resulting in egg cells, are widely used when referring to the process in a particular sex of an animal. In seed plants the formation of haploid cells, which eventually give rise to sperm nuclei in the pollen grain, is called *microsporogenesis;* its counterpart process producing female haploid cells is termed *macrosporogenesis*, or *megasporogenesis*.

The diagram given in Fig. 2 illustrates meiosis in an organism which has a diploid set of chromosomes numbering 4 ($2N = 4$). It will be noted that the chromosomes pair in the first stage of the first division (prophase I), thus, in this example, giving two sets of two chromosomes. The chromosomes which pair or *synapse* are described as homologues, because they can be shown to possess similar or identical sets of genes as well as being similar in appearance morphologically. It should be noted that the only way to demonstrate decisively the homology of two chromosomes is to note whether they pair in meiosis. The fact that they are similar in appearance and gene content is important, particularly the latter point to the geneticist, as will be discussed below, but the cytological fact of homology between chromosomes in a diploid is based upon the criterion of pairing rather than on genetic relationship, which is something quite outside the realm of pure morphological cytology.

At the time of pairing or shortly thereafter the chromosomes can be seen to have doubled in number by each member of a pair becoming duplicated. The result is the formation of a pair of closely associated duplicates from each chromosome which are distinguished from the mother chromosome by being called sister *chromatids*. Thus each original pair of homologues becomes a *tetrad*, or a packet of four *chromatids* consisting of two sets of two identical (sister) chromatids.

The members of each pair of sister chromatids remain attached to one another during this phase of meiosis because they have only one centromere (or spindle fiber attachment) between them, for when the sister chromatids are formed, the duplication process does not extend to the centromere, at least not to an extent that becomes evident.

With the formation of tetrads the prophase of the first division draws to a close and the second phase (metaphase) commences with the lining up of the tetrads in a single plane perpendicular to the spindle fibers, and approximately halfway between the poles of the cell. (See Fig. 2.) Metaphase ends with the homologous chromosomes separating and going to opposite poles. It will be noted from Fig. 2 that this is equivalent to the homologues *segregating*, for each daughter cell then receives a representative of each homologous pair present in the original $2N$ germ cell.

In the second division of meiosis the pairs of sister chromatids present in each of the two cells produced by the first division are broken up by the separation of the sister chromatids, which go to opposite poles. The net effect of this is the formation of four cells each with a haploid set of chromosomes. It should be noted by referring to Fig. 2 that each product of the meiosis receives a representative of each homologous pair. This is important. Gametes deficient in a chromosome type will generally not produce viable zygotes on fertilization. The second important point to note is that the segregation of homologues is *random*. Thus, if the homologues differ in gene content, from a diploid cell containing four chromosomes at least four different kinds of gametes result by meiosis. This can be readily appreciated by considering the combinations of *Aa Bb* by two's in which each pair contains one letter of each type, i.e., *AB*, *Ab*, *aB*, *ab*.

When chromosomes synapse in meiosis it can be shown that they do so with like parts being located opposite like. Hence, if chromosome *A* having genes *ABCDEFGH* arranged along it in that order were to synapse with its homologue *a*, which has the gene order *a b c d e f g h*, they would do so in the following way: $\frac{ABCDEFGH}{abcdefgh}$. Like genes, or allelic genes, as will be made clear below, are, in other words, located opposite one another. Failure of the apposition of like parts to take place results in absence of synapsis and the breakdown of the meiotic mechanism.

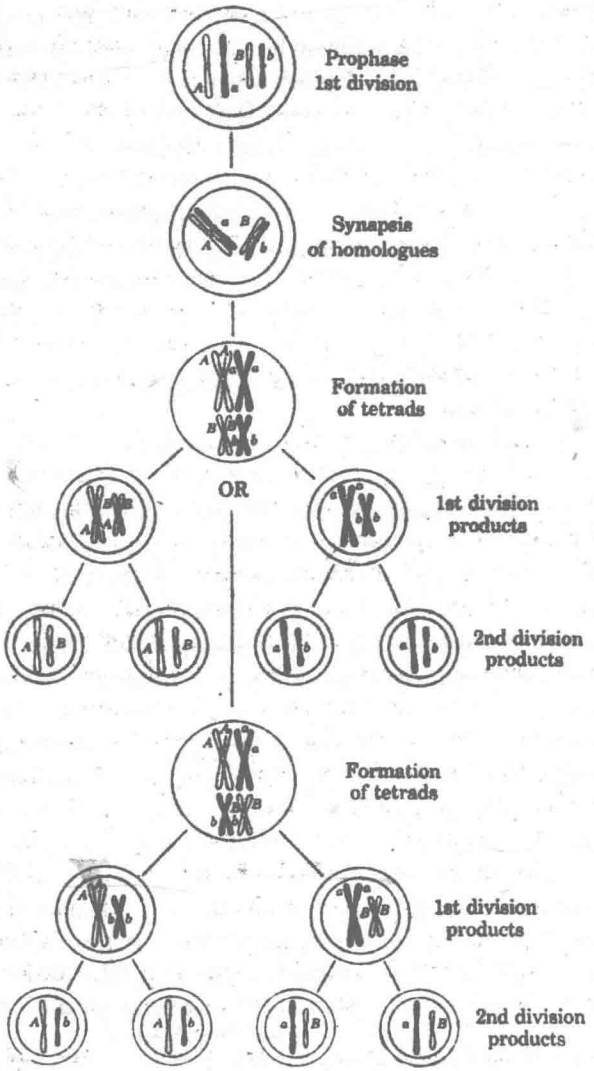


Fig. 2. A diagrammatic representation of meiosis. Meiosis is an extremely complicated process, and this figure attempts only to indicate how the products are derived. The process of crossing over illustrated in Fig. 4 and discussed on p. 11 is part of the process of meiosis, although not so indicated in this diagram.

3. Mendelism

The phenomenon of Mendelism is recognized by the occurrence of certain ratios of phenotypes of offspring. It is the direct result of meiosis with its random segregation in the parents, as described above, and of the random reconstitution of diploid offspring by fertilization, as described below.

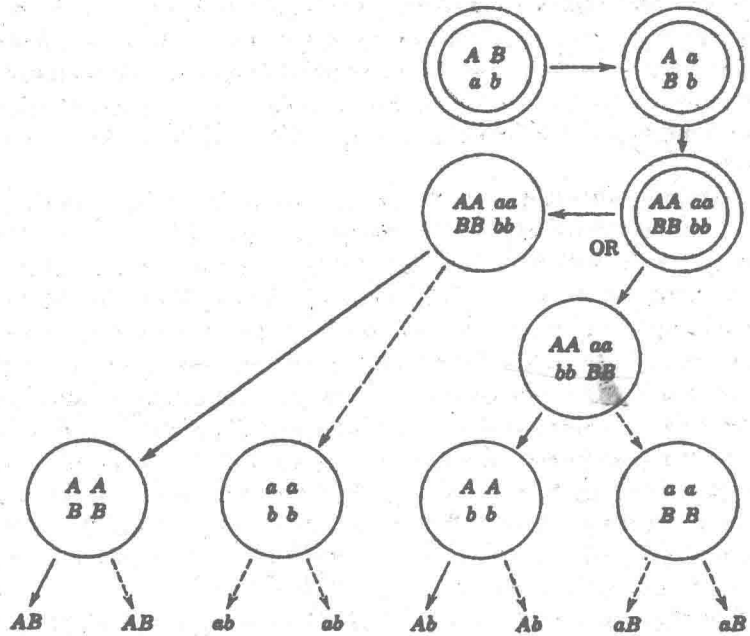
When an individual breeds true for a certain characteristic (i.e., inbreeding to its siblings or family stocks produces more of the same phenotype in the offspring without exception), it is *homozygous* for that characteristic. The term *homozygous* refers to the genotype. It means that an organism so described not only possesses the genes for the characteristic but also possesses identical kinds of genes on both sets of homologues. A homozygous individual produces, as the result of meiosis, gametes which are identical with respect to the genes for which it is homozygous. A homozygote when bred to another with the same genotype will produce offspring identical to the parents and one another. This statement may be written in genetic shorthand: $AA \times AA$ (where A denotes a gene giving a particular characteristic) gives in the F_1 (first filial generation) AA offspring.

Two individuals with different phenotypes when bred together very often give offspring identical to one of the parents. If both parents are homozygous for their respective genes controlling these characteristics, the cross may be written as $AA \times aa$. By this means it is stated that one of the parents, AA, is homozygous for A, and at the time of meiosis one pair of its homologues could be marked thus, $\frac{A}{A}$. The genotype of the second parent may be written $\frac{a}{a}$. Now, if these genes are assumed to occupy equivalent positions on homologous chromosomes and hence are alleles, it is evident that the gametes produced by parent AA will be A, those by parent aa will be a, and the F_1 offspring will be $\frac{A}{a}$ or simply Aa. These offspring are described as *heterozygous* for the genes A and a, which is another way of saying that they are not pure breeding, since they will produce two types of gametes, A and a, in equal numbers as a result of the segregation of the homologues in meiosis. If the F_1 offspring are inbred, therefore, three kinds of genotypes will appear in the F_2 generation, AA, Aa, and aa. As a

result of random fertilization of equal numbers of *A* and *a* eggs by equal numbers of *A* and *a* sperms, these three genotypes should occur in the ratio of 1*AA*:2*Aa*:1*aa*.

It was stated above that in this example the F_1 offspring are identical to one of the parents. Assuming that *Aa* heterozygous individuals are phenotypically identical to the parents designated genotypically as *AA*, then it is evident that the gene *A* masks the effect of its allele *a*. The more usual way to describe this effect is to call *A dominant to a*, or, to put it another way, call *a recessive to A*. The F_2 genotypic ratio of 1*AA*:2*Aa*:1*aa* can be expressed phenotypically as a 3:1 ratio, since *AA* and *Aa* individuals are phenotypically indistinguishable. The 3:1 ratio is a *Mendelian ratio* generally described as the ratio expected from a cross between two individuals heterozygous for a pair of allelic genes both of which have something to do with the determination of the alternative phenotypes. In short, it is a ratio which when obtained tells the breeder that two alternative phenotypic characteristics are produced by allelic genes. If one allele is not completely dominant over the other, incomplete or no dominance will result, and the heterozygote will be phenotypically distinguishable from the homozygotes. This condition will be manifested by a 1:2:1 phenotypic ratio identical to the expected genotypic ratio.

The 3:1 or 1:2:1 ratios are the basic Mendelian ratios. All other ratios are derived from them. Consider, for example, two animals of opposite sex which are heterozygous for two pairs of allelic genes, each pair located on a different chromosome, with the genotype *Aa Bb*. Figure 3 illustrates the types of gametes that would be expected from meiosis in each sex with details of how these gametes are derived. It will be noted that for each female diploid cell that undergoes meiosis only one of the four haploid products survives as a functional gamete. Oögenesis in animals is identical in principle to spermatogenesis with respect to the nuclear contents, but there is an unequal distribution of cytoplasm such that one nucleus retains to the end of the second division all or nearly all of the cytoplasm. This is the functional gamete; the others are polar bodies and disintegrate in time. Since, however, it is a random matter of chance which of the four nuclei resulting from the original diploid cell receives the cytoplasm, the same types of gametes should be expected in oögenesis as in spermatogenesis provided that the genotypes of the diploid germ cells are the same. The essential factor to be recognized in connection with meiosis involving more than a single pair of homologues is that it is a matter of chance which non-homologues accompany each other in the first di-



Female gametes

Male gametes	AB	ab	Ab	aB
AB	AABB	AaBb	AABb	AaBB
ab	AaBb	aabb	Aabb	aaBb
Ab	AABb	Aabb	AAbb	AaBb
aB	AaBB	aaBb	AaBb	aaBB

Fig. 3. The results of the segregation of two pairs of homologous chromosomes. The chromosomes illustrated in Fig. 2 are represented here by their letters. The dotted lines indicate the formation of polar bodies which occurs during oögenesis. As indicated in the text, the principles involved in the formation of male and female gametes are identical. The Punnett square at the bottom of the figure shows the different possible genotypes obtained by crossing two individuals who are heterozygous for genes on two different chromosomes.

vision of meiosis. Thus in Fig. 3, in a cell with *AaBb* marked chromosomes, *A* can accompany *B* and hence *a* will go with *b*, or *A* and *b* going to one pole will result in *a* and *B* in the opposite pole. Hence as pointed out above four types of gametes are expected from a cell heterozygous for two pairs of genes on different chromosomes. Three pairs of allelic genes on as many different chromosomes will result in eight different gametes.

The results of fertilization with the gametes of the genotypes shown in Fig. 3 are found by use of the Punnett square as illustrated. The genotypic ratio is of course complex because of the large number of different genotypes obtained. The type of phenotypic ratio obtained will depend upon the type of dominance relationships between the alleles and also upon possible interactions between the non-allelic genes. Such interactions need not concern us here but are discussed at length in Chapter 10. If complete dominance of *A* and *B* over their respective alleles and no gene interaction is assumed, a 9:3:3:1 phenotypic ratio will result. This may be written as $9AB$; $3Ab$:$3aB$:$1ab$, since *AB* may be taken to be the phenotypic designation of *AaBb*, *AaBB*, *AABb*, *AABB*, and so forth for the others. Other types of ratios resulting from gene interactions are given in Table 36, Chapter 10.

The fact that these ratios can be derived from the simpler 3:1 ratio obtained when but a single pair of allelic genes are segregating can be appreciated by the application of some simple probability. For example, in a cross of $Aa \times Aa$, the chances of an offspring having the gene *A* (that is being either *Aa* or *AA*) are 3 out of 4, or 3/4. There is a like probability that the offspring will have *B* from a cross $Bb \times Bb$. Since *B* and *b* segregate independently from *A* and *a*, the probability that any individual will have both *A* and *B* as a result of a cross $AaBb \times AaBb$ will be $\frac{3}{4} \times \frac{3}{4} = \frac{9}{16}$. Similar rearranging gives the other components of the $\frac{9}{16}$:$\frac{3}{16}$:$\frac{3}{16}$:$\frac{1}{16}$ ratio by starting with the 3:1 ratio.

It should be clear from the preceding discussion of Mendelism that if two organisms from pure breeding homozygous stocks, but of different phenotype, are crossed and the F_1 inbred, the phenotypic results in the F_2 will determine the genotypic nature of the phenotypic differences. A definite 3:1 or 1:2:1 ratio obtained in the F_2 indicates that the difference is *monogenic*, i.e., the result of the difference in expression between two allelic genes. If on the other hand a different ratio such as 9:3:3:1, etc., is obtained the conclusion must be that more than a single pair of allelic genes is involved. Hence it can be seen that the definition of the gene rests upon the kind of Mendelian ratio obtained,

and that furthermore the number of gene pairs involved, if more than one, in any phenotypic difference may be deduced from these ratios.

4. Linkage and Crossing over

Since each chromosome contains many genes arranged linearly, it is to be expected that a phenotypic difference may easily be the result of a difference in two non-allelic genes on the same chromosome. Such a situation is described as linkage. For example, assume that the genes

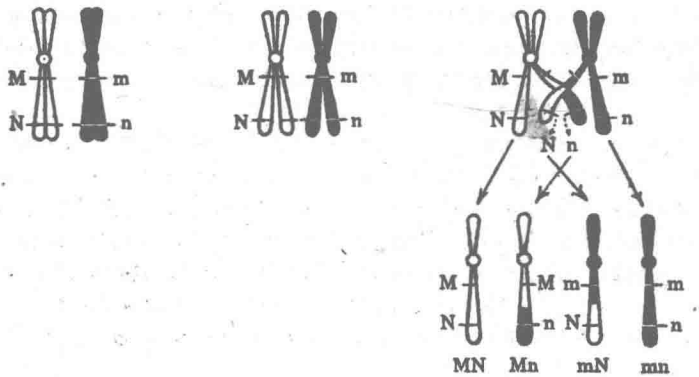


Fig. 4. Crossing over between the chromatids of a pair of homologous chromosomes.

M and N are located on the same chromosome and that m and n are their respective alleles. If two individuals, $MMNN$ and $mmnn$, are crossed, the F_1 genotype will be $MmNn$, or $\frac{M \quad N}{m \quad n}$, and, according to the information given in the preceding sections, the F_2 will consist of three types of progeny, $MMNN$, $MmNn$, and $mmnn$, in a 1:2:1 ratio. Since this is the ratio to be expected from the F_2 of a cross involving parents differing only in a pair of alleles, the conclusion must be that M and N are not recognizable as different genes. However, the integrity of the chromosomes, although maintained in substance from generation to generation, is not absolute. After the formation of chromatids in the first prophase of meiosis, and prior to the separation of the elements of the tetrad, the phenomenon of *crossing over* occurs. Figure 4 gives a diagrammatic representation of crossing over between two non-sister chromatids of a tetrad in the region between the loci of two genes M and N. The net effect of the crossover in this region is