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J. H. LONGWELL, *Director*

Gene Structure and Gene Function

In Memoriam
LEWIS JOHN STADLER
1896-1954

memorial lecture by
GEORGE W. BEADLE, CALIFORNIA INSTITUTE OF TECHNOLOGY

forewords by
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THE GENE

Dr. Stadler's last paper
Reprinted from SCIENCE

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COLUMBIA, MISSOURI

Lewis John Stadler
1896-1954

LEWIS JOHN STADLER

Late Professor of Field Crops,
University of Missouri

Geneticist,
U. S. Department of Agriculture

This publication is issued in commemoration of the late Lewis John Stadler, Professor of Field Crops at the University of Missouri and geneticist for the U. S. Department of Agriculture. Dr. Stadler died on May 12, 1954, after a prolonged illness. On February 3, 1955, the Missouri Chapter of Sigma Xi, in collaboration with Gamma Sigma Delta, the honorary society of agriculture, held a "Stadler Memorial Lecture" with Dr. G. W. Beadle as the chief speaker. This lecture on "Gene Structure and Gene Function" and Dr. Stadler's last paper on "The Gene" are presented in full.

It was thought appropriate and desirable to print also a foreword given at the lecture by Dr. H. E. Bent, Dean of the Graduate School, University of Missouri, and a biographical statement of appreciation of Dr. Stadler by one of his former students, Dr. John R. Laughnan, Chairman, Department of Botany, University of Illinois. A complete bibliography of Dr. Stadler's publications is appended.

A. E. Murneek, President
Missouri Chapter of Sigma Xi

June, 1955

CONTENTS

The Career of Lewis John Stadler

by John R. Laughnan, Chairman
Department of Botany, University of Illinois

Lewis John Stadler, geneticist extraordinary, died in Barnes hospital in St. Louis, May 12, 1954. During his career, the end of which is the more tragic for its brilliance, Stadler designed and executed genetic experiments which were remarkable for their scrupulous attention to detail and distinguished by the fundamental nature of the problems under investigation. The briefest of conversations with him sufficed to impress one with the gravity of his thoughts and the singular degree of mental and verbal acuity which he brought to bear on their expression. Many were the discussions in learned groups to which he lent orientation and improved focus and it is doubtful whether there are any geneticists who did not experience in his presence a serious challenge to their powers of imagination and reasoning.

Stadler came to the University of Missouri in 1917 from the University of Florida where he received his B.S. degree in Agriculture. His research activities during, and shortly after, his graduate years (1917-1922) at Missouri dealt primarily with breeding methods in corn and small grains and with test-plot design and error analysis as applied to these crops. Following these studies his research pursuits turned abruptly toward the investigation of more fundamental genetic problems, the first of which dealt with the variability of crossing over in maize, published in 1925 and 1926. At this time Stadler was engaged in the initial studies on the genetic effects of X-rays in barley and maize which were pursued concurrently with those by H. J. Muller in *Drosophila.* The co-discovery of the mutagenic effects of X-rays was hailed widely for its significance in both basic and applied areas in genetics, but Stadler's own inspiring researches over the next decade carried on primarily with maize, were to indicate that X-ray induced genetic changes in maize are extragenic in nature and are not to be confused with gene mutations. The subtle differences which in many cases distinguish the X-ray induced mutants as extragenic events are reminiscent of the anomalous situation which existed for so long in the cases of the *Oenothera* mutations. Under the scrutiny of one less gifted they could easily have gone unrecognized. It was these subtle differences whose elucidation required detailed and exhaustive analysis at specific loci which convinced Stadler that the statistical approach to the study of gene mutation carried on en masse at large numbers of loci is fraught with serious dangers of misinterpretation.

While Stadler retained a research interest in the problem of the nature of X-ray effects, the consistent recovery from this source of derivatives which could be excluded as having an extragenic origin led him and his coworkers to investigations of the genetic effects of ultraviolet radiation. With F. M.

Uber he showed that the action spectrum for genetic changes in the ultraviolet corresponds closely with the absorption spectrum of nucleic acids. With ultraviolet radiation he obtained rare mutations at specific loci which, under critical analysis, could not be distinguished from gene mutations. Even so, because of the preponderance of extragenic effects from this source, he was not convinced that these rarer types were in fact gene mutations. For this reason his research endeavors during the later years of his career turned toward intensive analyses of spontaneous mutation at specific loci, notably *A* and *R* in maize. His earlier published data on spontaneous mutation rates at certain selected loci in maize are classical and still widely quoted; yet his own later studies indicating that the mutation rate at the *R* locus is strikingly influenced by modifiers, the first evidence of its kind in maize aside from mutable gene systems, lent a more dynamic aspect to the problem of gene mutation.

In recent years, and at the time of his death, Stadler's attention was given to an analysis of the clustered genic elements at the *R* locus which, because they are associated with rare crossovers, are a confounding influence in the studies on spontaneous mutation. His final work, "The Gene," which he completed shortly before his death, is a summing up of his explorations in pursuit of the nature of this fundamental biological entry and of the elusive changes which it undergoes.

Perhaps because he was so persuasive in his arguments in support of fundamental research, geneticists are not generally aware of Stadler's contribution in the applied area. He was primarily responsible for the discovery and development of Columbia oats, which for two decades since its release in 1930 has been a superior oat variety in the southern Corn Belt. More recently, Stadler innovated the gamete selection method in the development of inbred lines of corn, a technique designed to take advantage of crosses to present elite lines in selecting superior gametes from varietal populations.

It is difficult to assess adequately Stadler's influence in genetics and elsewhere in scientific circles; moreover, an account of this sort directed to geneticists would be superfluous. My own high regard for his contribution to our science is rivaled only by the personal admiration I feel for him.

Dr. Stadler is survived by his wife, Cornelia Tuckerman Stadler, five sons, Tuckerman, Henry, David, John and Eliot, and daughter Joan.

Dr. Stadler's Contribution to University of Missouri

A Foreword by H. E. Bent,
Dean of the Graduate School
University of Missouri.

The magnitude of the contribution of Professor Lewis J. Stadler to the the program of the University of Missouri and especially to the Graduate School, makes most appropriate the recognition by the University in some formal way of its great debt to one of its distinguished scientists.

The life of Lewis Stadler reminds us most pointedly of the nature of a great University. In the hurry and worry, in the complexity and endless detail of modern life it is all too easy to forget the essential features which go to make up a great institution.

It is difficult to imagine the operation of the University of Missouri without a president and many administrative and business officials. Yet these do not constitute the real University. It is impossible to imagine a university carrying on without buildings and equipment, both books and scientific apparatus. But these are inert objects until brought to life by the touch of a human mind. It would be a strange institution which did not have a large body of students with their endless activities, social life, recreation, lectures and final examinations. But none of these constitute a great university or even a part of a great institution until it has a company of scholars composing an outstanding faculty.

Lewis Stadler reminds us that the real University of Missouri is essentially a company of scholars, that all administration, all buildings and books serve merely to make these scholars more effective. That all students who come to the campus for an education come here for the influence which faculty can have upon them—upon their abilities to observe and reason, to form judgements and choose values and to put these together in effective, creative activity.

These are the important functions of a university, but are slow to be achieved, and at no time are conspicuous. Hence, the importance of giving them the greatest possible emphasis by exemplifying their achievement in the life work of an individual.

Lewis Stadler's great contribution to the University of Missouri was the stature which he achieved as a scholar and as a scientist. No higher contribution to the University can be made by any individual. He was recognized as an authority in Genetics, not only on this campus but throughout the nation and the world. This is not simply a proud boast of the University of Missouri but is substantiated by the fact that he was called upon to serve

on national and international commissions. The National Research Council frequently called upon him to serve on important committees. His election to the National Academy of Sciences and to the Presidency of Sigma XI were awards of the highest honor. Participation in the International Congress of Genetics indicated the high esteem in which he was held by foreign scientists. Scientists frequently came to our campus for the purpose of discussing their research problems with one who was able to grasp quickly the significance of problems and difficulties outside his own field and to make constructive suggestions of great value. As a scientist his stature is seldom equalled.

The University of Missouri may well take great satisfaction in these achievements of a man who is almost unique in having been identified so continuously with one institution. True, he taught or studied for a short time at outstanding institutions such as the California Institute of Technology and Harvard University. But essentially Lewis Stadler was *of* the University of Missouri. He came here as a student and devoted almost his entire life to teaching and research on this campus.

More specifically, he acted as an important advisor for many aspects of our graduate work. For many years he was a member of the Board of Editors of The University of Missouri Studies. As Dean of the Graduate School, I am happy to express my own debt to his good judgment and unfailing willingness to render assistance in connection with the graduate program of the University. My need for counsel and advice very frequently took me to Lewis Stadler's office where I met with a cordial welcome and received the sound judgment and wise counsel which I came to expect from such visits.

Most conspicuously on the campus, we observe the program which Professor Stadler established in the field of Genetics. Only a handful of institutions can boast of comparable work supported by such able scientists as are at the University of Missouri. In any institution, the development of such a program is a great achievement. At the University of Missouri it is particularly noteworthy when for a number of years it has been difficult to receive the kind of financial support which would make such development easy. Only those intimately familiar with the organization and finances of the Institution can appreciate the competition for funds, the pressures for support of new programs as well as for those long established, and the limitations of finances which made the development of a new program next to impossible. Only by continued effort within the Institution and the attraction of outside financial support could this program have been accomplished. This achievement is a tremendous tribute to the perseverance of Lewis Stadler and the soundness of his research program. He left us with a building and equipment adequate for the support of important research, and a reputation not only to be proud of but also to serve as a high standard to stimulate our best efforts for the future.

Perhaps the most significant contribution which Professor Stadler made to the graduate program of the University of Missouri was in his clear perception of the proper role of research in a University program. With a steadiness of purpose seldom equalled, he pushed forward the development of the science of Genetics by work of the most fundamental character.

Although far from indifferent to human needs or the value of applied research, still he never allowed these distractions to interfere in any serious way with his major activity. In this he demonstrated his poise and good judgment when confronted with one of the distressing paradoxes of life, namely, how to remain sympathetic to human needs and the insistent demands for solutions for practical problems while at the same time keeping clearly in mind that ultimate success in the solution of all of our human problems depends upon keeping our eyes fixed upon a more distant goal.

His was a strong and abiding belief in the dignity of scientific investigation as the greatest expression of the human spirit, and a confident belief in the eventual importance of the development of the science of Genetics. This significance was never narrowly interpreted only in terms of dollars and cents or even in immediate increased human comforts, but more widely to include a richer intellectual life in the broadest sense for the entire community.

This emphasis of Lewis Stadler's is particularly significant at a time when society is placing great pressure upon investigators and when the need for immediate results tends to distort the normal growth of a science. These pressures come from many directions. At the moment, the most insistent pressure arises from the conflict of ideologies which places us in a peacetime state of preparedness never before experienced by our country. Added to this is the need to increase food production at a time when the world's population is increasing more rapidly than our ability to produce and we have an imposing force in the direction of diverting research to immediate results. Finally, there is the ever-present desire to justify to society the investment of large sums of money in fundamental research.

The total of these demands produces a compulsion which only the most resolute investigator with great confidence in his ability to produce important fundamental work can resist.

It is not possible to sum up in a brief statement the achievement of Professor Stadler. To a remarkable degree his whole life and all of his energies were devoted to his scientific activities. Only a description of his entire life would do justice to his achievement. The University of Missouri is fortunate in having had Lewis Stadler as a member of its faculty and will long remember his achievements and profit by his influence.

The central problem of biology is the physical nature of living substance. It is this that gives drive and zest to the study of the gene, for the investigation of the behavior of genic substance seems at present our most direct approach to the problem.

Lewis J. Stadler, 1954

Gene Structure And Gene Function*

A LECTURE DEDICATED TO DR. STADLER

by George W. Beadle
California Institute of Technology
Pasadena, California

Biology teaches that a living creature can be at least partly understood in terms of a large number of molecules and macromolecules intricately organized spatially and capable of taking part in numerous chemical reactions by which the integrity of its structure is maintained and by which it carries on its many life processes. Unlike a non-living system it has the ability to requisition selectively and systematically from a suitable environment a heterogeneous array of raw materials and a supply of energy which are used for its repair, operation, and reproduction.

Modern genetics holds that the primary guidance of the delicately balanced and elaborately interrelated processes of development and function comes from genetic material carried in the microscopic thread-like chromo-

*It is with a deep sense of humility that the author dedicates this lecture to the memory of Lewis J. Stadler. Science and the society of which it is a part owes much to him. He devoted a large part of his remarkable intellectual ability and energy to investigations on the nature of the hereditary material. His contributions are of great signiificance. He was critical in his judgments and impatient with intellectual looseness. In his writing and speaking he was clear and honest. He added to these basically fine qualities a friendliness, modesty and warmth that those who had the privilege of knowing him can never forget.

This published version of the Stadler Memorial Lecture has been considerably modified and added to as compared with that presented orally at the University of Missouri on February 3, 1955.

somes of the cell nucleus. What this material is, how it is reproduced, the manner in which it functions, the mechanisms of its transmission from one generation to another, and what its role is in the process of organic evolution are basic questions in the science of genetics (25). Progress in answering them has been so rapid in recent years that concepts fundamental to all of biology require revision.

The Austrian abbot, Gregor Mendel, who published the results of his studies on inheritance in the garden pea in 1865, laid the foundations of modern genetics. To account for varietal differences in this plant, he postulated a series of discrete hereditary units, now called genes.

The geneticists and cytologists who followed him—beginning with the "rediscovery" and full appreciation of his work in 1900—have shown that the rules he so clearly formulated apply in principle to many kinds of organisms. In fact, it now seems a reasonable assumption that if one allows for differences in life cycle, the hereditary material is basically similar in all living things—peas, corn, flies, molds, protozoa, bacteria and even viruses.

In all of these forms the hereditary material is arranged in microscopic or even submicroscopic thread-like bodies called chromosomes.

In maize *(Zea mays),* to take as an example the organism with which Stadler worked, there are present in nuclei of all cells of the plant ten kinds of chromosomes. In the cells of the sporophyte of corn (the plant we see), there are two sets of ten. In spore formation, the number is reduced from two sets to one by two special cell divisions known as the meiotic divisions. Gametes—egg and sperm nuclei—also contain one set each. On union of egg and sperm nuclei at fertilization, two sets of ten again come together in a single nucleus.

In each chromosome there are many genes, probably hundreds or thousands, each of which appears to occupy a fixed and special position known as a locus. These positions are defined in two ways. The order and spacing of the genes in a single chromosome is found by techniques of detecting linkage and measuring recombination frequencies that are so simple in principle they are regularly taught in beginning biology courses. That these positions are correct and referable to chromosomes as seen under the microscope can be verified by slightly less simple methods of correlating visible chromosome abberations—such as deficiencies, duplications, translocations and inversions—with genetically detectable aberrations in gene behavior. For example, absence of a specific small segment of chromosome can sometimes be causally related to absence of a specific gene.

The existence of a specific gene can be inferred genetically only if it exists in two forms (alleles) that have detectably different effects on the organism. In maize, for example, the *R* gene is known to be necessary for the formation of a purple anthocyanin pigment in the endosperm of the kernel

because this pigment fails to appear if in all cells of the endosperm of the kernel the *r* allele is substituted for its dominant counterpart. A study of the linkage relations of the *R* gene shows it is always located in a specific place in the tenth chromosome.

Like many other genes, the *R* allele is known to be mutable (31). If thousands of descendants of this allele are investigated in a suitable manner an occasional instance will be found in which *R* has apparently spontaneously changed to an *r* allele. This particular mutant change lends itself to investigation because it is detectable in the seed stage and hence many thousands of individuals can be observed for possible mutations. The frequency of the change varies from strain to strain.

So far we have used the term gene rather loosely. Can we define it precisely? This is a question to which Stadler devoted much attention. As we shall see the answer is not easy to arrive at although there are several ways of approaching it.

The Gene as a Unit of Crossing Over

The *R* gene can be defined in terms of its location in the 10th chromosome. In this way it can be distinguished from other genes that influence anthocyanin synthesis in the endosperm. Thus the gene *C* is also essential for the formation of this pigment in the endosperm. If its recessive allele *c* is substituted for *C*, no anthocyanin pigment is produced. *C* can be shown to be located in chromosome 9. Hence if both *R* and *C* are segregating in a single plant, they do so independently. By virtue of their different locations they can be said to be different genes. The same experimental method can be used to differentiate genes within a single chromosome, even when they are only a fraction of a crossover unit apart. On this basis genes can be said to be entities that behave as units in recombination, whether they segregate independently or are linked.

The Phenotypic Tests for Allelism

If one has two strains of maize with no anthocyanin in the endosperm, both differing from a colored strain by a single recessive gene, the question of whether they differ from the colored strain by the same or different genes can be determined by crossing the two colorless lines. If they both carry recessive alleles of the same gene, the first generation hybrid is expected to be colorless. But if they carry recessive alleles of different genes, a colored seed is expected to be produced in the first generation. Using genes *R* and *C*, these two results are symbolized as follows:

Alleles: *CC rr* (white-1) x *CC rr* (white-2) → *CC rr* (white)

Separate genes: *CC rr* (white-1) *x* *cc RR* (white-2) → *Cc Rr* (colored)

Although there are notable exceptions, this test usually gives the same answer as the location test—that is, genes occupying different loci are not allelic by the phenotypic test for allelism, whereas those found by the position test to be at the same locus are alleles.

Multiple Alleles

Early in the history of genetics is was discovered that some genes can exist in several allelic states. A classical case is that of white eye in the fruit fly Drosophila. There are a series of recessive eye-color mutants, ranging from the dark red of the wild fly to white, that by both the location and allelism test are differentiated from the normal red eyed fly by different alleles of the same gene. These alleles can be arranged in a graded series both with respect to eye pigmentation and dominance. Alleles that give more pigment are dominant to those with less. But no combination of two mutants gives complete wild-type pigment.

As more refined methods of detecting differences in end effects become available, so many examples of multiple alleles are found that one is tempted to generalize that all genes can exist in many allelic states.

The *R* Locus

Early in his studies on plant pigment inheritance, Emerson (9) found that there exist at the *R* locus different states of the genetic material that in some ways behave as different genes and in other ways as multiple alleles.

In addition to the *R r* pair of alleles concerned with kernel pigmentation, there was found a closely related pair of alleles concerned with anthocyanin formation in the stem and certain other parts of the plant. When the simple allelic test is made by crossing a plant with green stems and purple kernels with one possessing red stems and white kernels, the first generation kernels are purple and give rise to red-stemmed plants. This suggests separate genes for pigmentation in the two parts of the plant.

But early location tests showed that both genes are in the same place in the chromosome and either do not separate by crossing over or do so with a frequency so low as to be experimentally difficult to detect. Thus the hybrid between purple-seeded, green-stemmed and white-seeded, red-stemmed strains produced only two types of gametes, not four as would be expected if two genes were involved. The recombinant gametes expected (for color in both or neither of the two plant parts) if the two genes were located in separate positions, were apparently either very rare or did not occur.

In spite of this absence of crossing over there are found among the many varieties of maize that have been investigated the combination in which both seeds and stems are colored and that in which both are colorless. In these also the two pairs of genes are inherited as units.

In general it is found that the several alleles of a single multiple allelic series are concerned with the same character. Partly because of this and partly because little or no crossing over was found between seed and plant color, the genes concerned were treated as multiple alleles and given the following symbols:

	Kernel	Stem
R^r	colored	red
R^g	colored	green
r^r	colorless	red
r^g	colorless	green

Actually, several additional states of the locus are known and these too have been interpreted as being *R* alleles. We see, therefore, that the use of the r^g allele in the phenotypic test for allelism with R^g and r^r results in an interesting ambiguity. R^g/r^g has colored endosperm and green stems, thus indicating allelism. r^r/r^g has colorless endosperm and red stems, again indicating allelism. But it will be recalled that the R^g/r^r combination has color in both endosperm and plant stem, thus indicating separate genes. All results are formally consistent with the assumption that there are two closely linked but non-allelic genes with r^g being inactive for both, i.e., a double mutant.

In part because of results like those just described Stadler selected the *R* locus as one of several that he and his associates investigated in detail in their attempts to learn more about the nature of the genic material. Some of the results will be summarized below.

Gene Mutation

One method of finding out about genes is through a study of their mutational properties. The frequency with which genes mutate spontaneously is variable from species to species, from one genetic type to another within

a species, from gene to gene within a species, and from one environment to another for a given gene in a particular species. The development of methods of measuring mutation rates quantitatively was an important step in genetic studies. H. J. Muller and Stadler were pioneers in this; Muller used Drosophila as experimental material and Stadler used maize.

Stadler was one of the first to measure spontaneous rates of mutation for specific genes. For this he used a series of genes affecting endosperm characters. In this way he was able to obtain the necessary large populations without inordinate labor and expense. Recently, methods have been developed for measuring mutation rates in microorganisms in which, by using enrichment culture techniques, even larger numbers can be used than with flies or maize.

Excluding so-called unstable genes, spontaneous mutation rates are found to vary from more than 1 mutation in a thousand genes per life cycle to less than 1 in a billion.

It has now been known for more than a quarter of a century that mutations can be greatly increased in frequency by high energy radiation. This finding, which opened the way to many significant advances in genetics, was made independently and almost simultaneously by Muller and by Stadler. More recently it has been found that mutation frequencies can be increased by treatment with a number of chemical substances (8).

The results of early experiments on the mutagenic effects of high energy radiation were interpreted at first in terms of the so-called single hit hypothesis—that ionization or excitation in or very near a gene was necessary and sufficient to induce a mutant change. This interpretation, plus a consideration of the observed effect of temperature, leads to the elaboration of a physical model of the gene. In this there were two important sources of error that initially were not recognized.

First, it is now known that genetic effects of radiation are at least partly indirect. X-rays and ultraviolet, for example, produce extensive formation of highly reactive free radicals throughout the protoplasm of the cell. These may then produce secondary effects including changes in genes. A second source of error that has been particularly stressed by Stadler (33) is the fact that many of the mutations produced by ionizing radiation involve mechanical rearrangements of the genic material. Many of these—large translocations, large inversions and large deficiencies, for example —can be detected fairly readily genetically or, in favorable material, by cytological examination. As Stadler has insisted, however, there is at present no known way of distinguishing such mechanical effects from true gene mutation, especially in those cases where the former are very small. The experimental evidence in maize, the life cycle of which is very favorable for screening out certain categories of mechanical rearrangements, suggests that very few or perhaps no

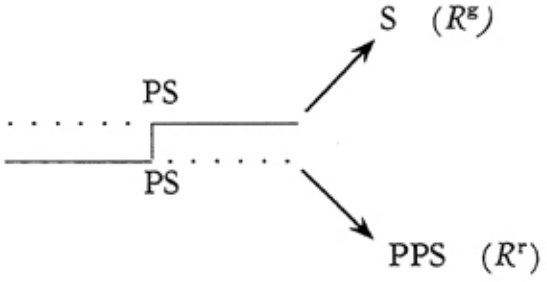
gene mutations of the kind that involve slight qualitative changes are produced by X-rays. Evidence from other organisms does not seem entirely consistent with such an extreme view but Stadler's point is nevertheless well taken that there is no experimental way known at the present time of distinguishing true gene mutation from less subtle mechanical effects.

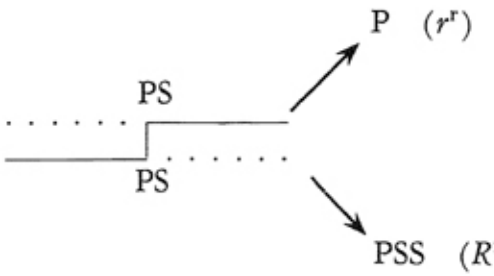
For these reasons Stadler emphasized the desirability of studying mutations that arise spontaneously.

Mutation Studies on the *R* Gene

If at the *R* locus there are two genes so closely linked that they rarely separate by crossing over, one concerned with plant pigmentation and the other with pigment formation in the endosperm, they might well be expected to show independence as regards mutation. With qualifications Stadler (31, 32) found this to be the case. If spontaneous mutations of the R^r allele are obtained in appreciable numbers, it is found that they are to r^r or R^g alleles in the great majority of cases, not to r^g.

This suggests separate units that are independent in their mutation. But in a further experiment in which crossing over was followed between markers on the two sides and close to the *R* locus at the same time the *R* mutants were detected (34, 35), the significant finding was made that R^r mutation to R^g or to r^r is frequently accompanied by crossing over at or near the *R* locus. Stadler and his coworkers have interpreted this in terms of a model in which R^r consists of two adjacent units, called P (for plant color) and S (for seed color), that are sufficiently closely related structurally and oriented properly to permit pairing and crossing over between them. Although both are somehow related to anthocyanin synthesis, they cannot be functionally identical because they are active in different parts of the plant. The crossover mutations of R^r to R^g and r^r are postulated to occur by process of unequal crossing over as follows:





If this interpretation is correct, the S crossover mutant (R^g) should not be able to give an r^g mutant by crossing over with PS (R^r) but the $R^r \longrightarrow r^r$ mutant should occur by crossing over in the same combination. Limited results so far reported from experiments designed to test this prediction are consistent with the hypothesis (34).

Because not all mutants of R^r are accompanied by crossing over it seems necessary to assume that both P and S are capable of mutating to the so-called "null" forms *p* and *s* in a conventional gene mutation manner, i.e., without crossing over (33).

A somewhat similar situation has been known for many years in the case of unequal crossing over at the Bar locus in Drosophila. This phenomenon was postulated by Sturtevant on purely genetic grounds and later verified cytologically by Bridges. In the original Bar mutant, which arose in an experimental culture, the duplicate segments are presumed to be identical. But through a subsequent genetic change, one of the segments became modified through what is assumed to be point gene mutation. Thus in the secondary mutant, known as Infrabar, the duplicate segments are functionally somewhat different but still synaptically equivalent.

A second locus in maize that is in some ways like the *R* locus has been investigated in detail by Laughnan (20), a former student of Stadler's. Using essentially similar methods, Laughnan has postulated that at this locus there are two segments, called α and β, concerned with pericarp pigmentation and with anthocyanin formation in the endosperm and other parts of the plant. In this case, too, unequal crossovers occur with a frequency of about one per thousand chromosomes.

In all of these cases, thorough study of the mutation process has added significantly to our knowledge of the hereditary material. The question as to how general are cases of this kind in which tandem duplications permit unequal crossing over cannot be answered at present. In addition to the Bar tandem duplication, there are several cases in Drosophila that are known or suspected of being similar in principle. All of these arose in the laboratory as mutant types. This plus the fact that the *R* and *A* loci were initially selected for study because of their unusual properties, suggests that tandem duplications that give unequal crossing over may not be of frequent occurrence in normal populations.

Pseudoallelism

There are found in Drosophila and a number of other organisms (21,22) instances in which genes with what appear to be closely related functions lie very close together in the chromosome but are still separable by crossing over. Unlike the *R* and *A* loci in maize they do not give unequal crossing over. The members of certain such clusters have been called position pseudoalleles by Lewis, who interprets them in terms of separate genes, the functions of which are related in a way that requires them to be physically close together. For this reason if mutant pseudoalleles that are contiguous are carried in the separate chromosomes of a pair, they result in a mutant phenotype. Thus if *A* and *B* are pseudoalleles of this type, the so-called trans-double heterozygote, $\frac{a\ B}{A\ b}$ will be of a mutant appearance, while the cis-genetic type, $\frac{A\ B}{a\ b}$, is often normal or more nearly so than is its trans-counter part.

In Drosophila in which somatic pairing of chromosomes normally occurs the *A* and *B* pseudoalleles in the trans-configuration can be still further separated if somatic pairing is reduced by a chromosome rearrangement. When this is done, the deviation from normal of the trans-type double heterozygote is often increased.

In the bithorax series of pesudoalleles, Lewis has demonstrated five units separable by crossing over. Experimental estimates of the map distances between adjacent members give values of 0.003 to 0.01 units, i.e., in normal flies one crossover is expected per 10,000 to 33,000 chromosomes.

It is postulated that the normal alleles of the five members function in the control of an equal number of steps in a sequence of reactions necessary for normal development. Each reaction is assumed to require as a reactant the product of the immediately preceding reaction in the chain. All reaction products except the final one are believed to be labile or limited in diffusibility to the extent that to serve as a substrate for succeeding reactions the next reaction in sequence must occur in the immediate vicinity. Thus if the gene that controls the reaction by which a given substrate is transformed does not lie adjacent to the gene responsible for its production, the over-all reaction sequence will be slowed down enough to interfere with the development of a normal phenotype.

How frequent are such pseudoalleles within a single species and how widely distributed are they among different groups of organisms?

In Drosophila, a total of about ten cases of what appear to be pseudoalleles are known or suspected (22). Even in the series of alleles of the white eye gene, mentioned earlier as the classical textbook example of multiple

alleles, there is now evidence that there are at least two elements separable by crossing over. As in other cases mentioned, there are two interpretations possible: the one, advocated by Pontecorvo (28), that the white gene is a single functional unit capable of mutation at two or more sites separable by intragenic crossing over, or that of Lewis which postulates two or more physically adjacent genes that are functionally closely related but separable by intergenic crossing over.

In at least half a dozen other organisms, including higher plants, fungi, and mammals (22) gene clusters are known that in one or more significant respects suggest pseudoallelism. As we shall see later new methods have recently been developed for the experimental study of the phenomenon in bacteria and viruses.

Pseudoalleles of this type may well be related in origin to the tandem duplications discussed in the previous section, but they differ in that they are no longer synaptically equivalent and hence do not give unequal crossing over.

The Lewis interpretation assumes that the members of a pseudoallelic group are separate genes in the sense that they are separable by crossing over or chromosome rearrangements, that they are not equivalent synaptically, that they are independently mutable, and that they possess different functions. Their only special property is that their cooperative action requires close physical proximity. It is this property that causes the phenotypic test for allelism to break down when it is applied to them.

According to the interpretation advocated by Pontecorvo and others, pseudoallelic clusters of the kind just discussed represent mutational changes at different levels in a single functional unit. On this basis the mutant nature of the trans-heterozygote requires no special explanation for it assumes that no normal allele of the gene in question is present. This hypothesis preserves the validity of the phenotypic test for allelism but requires that crossing over within the gene be possible. It forces one to make an arbitrary choice as to whether to define the gene in terms of function or in terms of units indivisible by crossing over. As will be made clear later the same option is presented by experimental evidence on gene structure derived in other ways. It is entirely possible that for different situations both interpretations may be correct.

From the standpoint of the origin of new genic material in a species, Bar, the *R* alleles, and the position pseudoalleles of Lewis might well be representative of three stages in an evolutionary series. Starting with a tandem duplication like Bar in which the component parts are functionally and synaptically alike, one can readily visualize gradual divergence in function through mutation in the duplicate segments to the stage represented by the *R* case. Such duplications provide a mechanism by which new genically controlled functions can be acquired through mutation without the sacrifice

of existing ones. Finally, it is not unreasonable to expect synaptic incompatability to arise either through accumulation of gene mutations or structural differences in the two segments.

Gene Function

Before considering further the question of gene structure it will be advantageous to consider briefly the matter of gene function. Early in the century it was several times suggested—for example, by Bateson, Garrod, Moore, Hagedoorn, Goldschmidt, Troland, Haldane, Muller and Wright—that genes might function directly as enzymes or by producing enzymes.

One of the earliest cases of the genetic control of a specific chemical reaction was that of a rare metabolic disease in man called alcaptonuria. In affected individuals alcapton (2,5-dihydroxyphenylacetic acid) is excreted in the urine (12). The disease seems to be inherited as a simple recessive. In the absence of the normal allele of the gene concerned, the initial step in the series of reactions by which alcapton is oxidized through aceto acetic acid to CO_2 and H_2O fails to take place, presumably because of lack of activity in a specific enzyme. The evidence suggested a gene-enzyme-chemical reaction relation to Garrod but the interrelations could not be more clearly defined because little was known at the time about either the nature of genes or that of enzymes.

Subsequently many additional instances were discovered and described in which similar relations appeared to hold. These included such processes as anthocyanin formation in plants, melanin formation in mammals, oxidation of xanthophyll in mammals, eye pigment formation in insects, atropinesterase activity in the rabbit, amylase activity in the silkworm, linamerase activity in clover, and a number of others. Systematic studies of strains of molds, bacteria and other microorganisms with genetic defects in ability to synthesize such essential metabolites as vitamins, amino acids, purines, pyrimidines. etc. have indicated that a great many biochemical reactions are normally dependent on the activity of specific genes.

As a generalization from investigations of the many metabolically defective mutant types that differ by single genes from the type from which they arose and that can be interpreted in terms of lack of activity of a particular enzyme, the so-called one gene-one enzyme hypothesis was formulated (17). This holds that aside from duplicating itself, the activity of a given gene consists solely in directing the specificity of an enzyme. Or in a more general form, the hypothesis holds that genes are unifunctional and serve to determine the specificities of macro-molecules such as proteins.

No attempt will be made to review the evidence for and against the unifunctional hypothesis of gene action. This has been done elsewhere (6, 13, 17). Regardless of whether it is correct or not—or is a much oversimpli-

fied but basically correct view—this hypothesis has abundantly demonstrated its usefulness in experimental work in chemical genetics.

Genes and Hemoglobin

An example of a gene in man that appears to function in controlling a particular protein is that concerned with sickle cell disease (26, 27). In this disease, largely confined to populations in central Africa, southern India and a region of Greece, or descendants of these peoples, there is present an abnormal form of hemoglobin (called S) that differs from normal hemoglobin (called A) in its electrophoretic mobility (27). In terms of their adult hemoglobins the following three genotypes are known:

s s	normal A hemoglobin
S S	mainly S hemoglobin
S s	both A and S hemoglobin

S hemoglobin appears to possess 2 to 4 less free carboxyl groups per molecule than does A hemoglobin (30).

Since *S S* individuals do not often live to reproduce unless given very special medical care, including rather frequent blood transfusions, there is strong selection against the *S* allele. Until recently, therefore, it was difficult to understand how the incidence of *S s* individuals could be as high as 40% in certain tribes of central Africa. If mutation from *s* to *S* were responsible, the rate would have to be very high in certain small populations and very low elsewhere.

The explanation now appears to be that *S s* individuals are more resistant to malaria than are normal *s s* persons. Allison (2) and others have demonstrated this experimentally and have shown that there is a close correlation between incidence of *S s* individuals in a population and presence of malaria in the region in which the population lives. Although it cannot be accurately determined what the frequency of *S s* individuals was among African negroes brought to the United States in slave days, the present incidence is reduced by approximately the amount expected on the basis of estimates of the original incidence, extent of outcrossing, and number of generations in a malaria-free environment.

It thus appears that in malarious regions the *S* allele has a selective advantage in the heterozygous form that balances its disadvantage in homozygous form. In other words, in each generation the excess deaths among *s s* individuals from malaria are assumed to counterbalance the reduction in frequency of *S* alleles in the population resulting from the death of *S S* persons from sickle cell anemia. The *S* mutant gene is thus favorable in malarious regions and unfavorable in the absence of malaria.

Why cells containing S hemoglobin provide an unfavorable environment for the protozoan that causes malaria is not known. It may well be that

the same factor that results in the distortion (sickling) of the red cells of *S s* individuals at low oxygen tensions makes these cells unfavorable for the entrance, survival, or multiplication of the protozoan.

Sickle cell disease is of particular interest genetically because it indicates that the gene concerned determines the charge in the hemoglobin molecule. Experimental evidence indicates that it is the protein part of the molecule that is affected, but it is not yet known with certainty whether S and A hemoglobins differ in amino acid composition or only in physical configuration.

There are now known in man half a dozen abnormal hemoglobins associated with various hereditary anemias (5, 19). Because hemoglobin is readily obtainable in large quantities, is easily prepared in pure form, and is relatively simple to characterize by such properties as crystal structure and electrophoretic mobility, these hereditary traits provide particularly favorable material for characterizing molecules that may well be directly dependent on gene action. Unfortunately genetic investigations in man are not easily made. As a result, it is not yet known how the various anemias are genetically related. Knowledge of this kind will be of very great significance in increasing our understanding of protein synthesis and structure in relation to the gene.

Tyrosinase Activity

Melanin, a high polymer pigment widely distributed in the plant and animal kingdoms, is formed through a series of oxidative reactions from the amino acids phenylalanine and tyrosine. In the oxidation of tyrosine to its 3,4-dihydroxy analogue and subsequently in the reaction sequence by which melanin is formed, the enzyme tyrosinase serves as a catalyst.

In animals, melanin serves as a screening pigment and in some cases is clearly important in protective coloration. In many animals, including man, albino forms are known in which melanin is absent from hair, skin, and eyes. Often albinism is inherited as a simple recessive genetic trait. The pink-eyed white rabbit is a good example. Pigmented breeds crossed with albinos give pigmented first generation hybrids which show segregation in the subsequent generation in the classical mendelian manner.

As in a number of other species in which melanin pigmentation has been studied genetically, the albino gene in the rabbit exists in several allelic states. In addition to an allele for full pigmentation and one for complete albinism, there is a third allele, recessive to the full pigmentation allele, which has the phenotypic effect of determining that the pigment-forming process will be temperature sensitive. The so-called Himalayan breed is pure for this allele. In animals of this breed pigment does not form at normal body temperatures but is produced at temperatures a few degrees lower.

As a result, the extremities of a Himalayan rabbit—nose, ears, feet and tail—are pigmented whereas the compact parts of the body are not. A similar situation exists in the Siamese cat.

A clue to the explanation of the temperature sensitivity of the pigment-forming system of the Himalayan rabbit and the Siamese cat is found in the mold Neurospora. In this organism melanin is formed. There is known a strain in which, like the Himalayan rabbit, pigment formation shows a strong temperature effect. Temperature lability of the process is genetically differentiated by a single gene from temperature stability (16).

Cell-free tyrosine-containing preparations of the two strains of Neurospora show quite clearly that their different responses to temperature can be referred to different temperature stabilities of their tyrosinases. The tyrosinase of the temperature-sensitive mutant is much less stable *in vitro* at 35° C. than is a similarly prepared extract of the normal strain.

Here, as in the case of normal and sickle cell hemoglobins, qualitatively different proteins are presumed to be specified by two different alleles of the same gene.

An analogous case is known in the bacterium *Escherichia coli* in which a mutant strain is known in which the vitamin pantothenic acid is synthesized normally at low temperatures but at a low rate or not at all at a higher temperature (23). Here, too, an enzyme with abnormal temperature sensitivity has been implicated.

Chemical Nature of Chromosomes

The chromosomes of higher plants and animals are known to consist of desoxyribonucleic acid (DNA) and protein bound together in the form of nucleoprotein. Direct chemical analyses of isolated chromosomes, staining reactions of chromosomes, ultraviolet absorption, and digestion experiments with the enzyme desoxyribonuclease or with proteolytic enzymes bear out this conclusion.

Recently Mazia and his associates (24) have shown that following treatment with metal chelating agents such as citrate, chromosomes placed in distilled water separate into segments about 4000 Angstrom units long. The suggested interpretation is that these units are normal structural parts of chromosomes and that *in vivo* they are held together linearly by bridges of divalent magnesium and calcium ions. It is further suggested that these units may represent individual genes and that crossing over somehow occurs at the calcium-magnesium ion connections that normally hold them in a fixed linear order.

Action Spectrum of Mutation

Indirect evidence consistent with the assumption that nucleic acid is a part of the genic material, or important to it, comes from experiments in

which the effectiveness of different wave lengths of ultraviolet radiation in producing mutations is measured. One of the earliest and most careful experiments of this kind was made by Stadler and Uber (36) using corn pollen as the treated material. After rather elaborate corrections were made for ultraviolet absorption by the cell wall of the pollen grains and the cytoplasm lying between the source of radiation and the sperm nuclei, it was found that per unit of incident energy, the wave-length effectiveness in producing mutation followed as closely as could reasonably be expected, the ultraviolet absorption spectrum of desoxyribonucleic acid, with a peak near 2600 Å.

These studies indicate that the energy of ultraviolet effective in producing mutations is absorbed by DNA.

Transforming Principles

A more direct indication that DNA is an important gene constituent comes from knowledge of so-called transforming principles. Evidence for these was first obtained in experiments on type transformations of pneumococcal strains (3).

Essentially the experimental evidence is this:

The pneumococcus (Micrococcus) that causes lobar pneumonia in man, and is virulent to mice, is found in many serological types. Type specificity is based on differences in antigenic properties of the polysaccharides that encase the bacterial cells.

Virulence is associated with presence of a capsule, for all strains that lack a capsule also lack virulence. Virulent strains occasionally mutate spontaneously to forms that lack a capsule and are avirulent. Such strains can be distinguished from their virulent parent by the appearance of the colonies they form on a suitable artificial medium—capsulate strains are called "smooth", acapsulate ones are designated "rough".

If a rough mutant originally derived from a smooth of serological type II back mutates to smooth, as it sometimes spontaneously does, the reverted smooth is normally of type II like the original smooth from which it came. But if the mutation back to smooth occurs in the presence of a very little DNA prepared from a smooth strain of type III and under other conditions that are reproducible, the smooth may now be of type III. The significant factor in "directing" the mutation is the DNA. This can be made as pure as it is possible by the presently available methods of enzyme chemistry and physical chemistry.

Corresponding to each type specific strain from which such DNA directing preparations have been made there apparently is present a specific DNA.

Transforming principles have now been demonstrated in pneumococcus for a series of characters such as amount of capsule formed, resistance to

penicillin, resistance to other antibiotics, ability to utilize specific sugars, and presence of specific proteins. Each of these is a specific DNA. The phenomenon has also now been demonstrated in other bacteria.

A simple interpretation of the experiments on transforming principle assumes that DNA is the material responsible for gene specificity and that when a loss mutation occurs, such as smooth type III to rough, the specific DNA associated with type III polysaccharide is lost or inactivated. In the process of transformation, this defective DNA is replaced from the outside by a genetically homologous active DNA.

This interpretation is strongly supported by experimental evidence of Ephrussi-Taylor (10) who has shown that for some characters there are series of mutually exclusive DNA units, corresponding to allelic forms of a single gene, whereas for other characters DNA units show all possible combinations as do independent genes. In studies of the incidence of simultaneous transformations by pairs of "non-allelic" DNA units, Hotchkiss and Marmur (18) have reported what appears to correspond to genetic linkage for one particular pair.

Bacterial Viruses

Identification of DNA with the hereditary units of the geneticist indicated by the evidence from transforming principles is supported by an impressive body of evidence from recent investigations of viruses (bacteriophages) parasitic on the colon bacillus *Escherichia coli* (14, 15).

Before presenting this it is desirable to review briefly the significant points of the life history of one of these viruses. The ones designated T2 and T4 will serve as examples. These are tadpole-shaped structures with polyhedral heads about 800 Å long and a tail slightly longer. The head consists of a protein coat and a desoxyribonucleic acid core. The DNA core can be removed from the coat by osmotic shock (1).

If virus and susceptible bacteria are placed together in a suitable medium, the virus particles are adsorbed to the surface of the bacterium by the tips of their tails. Presumably the initial step in this attachment involves complementary patterns of electrostatic charges on the bacterial surface and on the tips of the virus tails. These are believed due to ionization of free NH_2 and COOH groups of proteins (29).

Shortly after adsorption, infection occurs by injection of viral DNA into the host, the virus particle acting as microsyringe. This is shown by experiments in which viral protein is labelled with radioactive sulfur or phosphorous, S^{35} or P^{32}, by growing the viruses in host bacteria which were in turn grown on labelled sulfate or phosphate (15). After infection protein coats can be knocked off the bacterial cells in a Waring blendor and separated from the cells by centrifugation. If the protein is labelled with S^{35} in

such an experiment, the radioactivity remains with the coats. But if the DNA carries a P^{32} label, the radioactivity goes with the bacterial cells.

After injection of viral DNA into the host cell, there is a latent period of 10 to 15 minutes during which viral DNA is reproduced as much as several hundred fold. Thereafter viral coats are fabricated of protein synthesized by the host cell but of a kind not made by an uninfected bacterial cell. After 20 to 30 minutes the host cells are lysed with the liberation from each of some hundred infectious virus particles. If uninfected bacteria remain, the process is repeated.

Bacterial viruses are mutable in several respects. Mutant strains differing from the parental strain in host range occur spontaneously and can be selected by growing a large population of viruses on bacterial cells resistant to the parental type of virus. Types showing characteristic differences from the parental form in size of plaques (clear circular colonies) formed in a layer of bacteria growing on the surface of an agar nutrient medium.

That mutant strains of virus differ from their parents genetically is easily demonstrated by infecting single host cells with two mutant strains of the virus. If the two viruses carry genetically different mutants, recombination types occur although the "mating" process in viruses is in some ways less simple than that in higher forms. The frequency of recombinations indicates genetic map distance apart of the two genes. In this way many genes have been mapped in T2 and T4.

Obviously if bacterial viruses have genes, and only viral DNA enters the host cell, genes of the virus must consist of DNA.

In multiplying inside the host, viral genetic material must use the metabolic machinery of the bacterial cell as a source of energy and the substance of the bacterium as building material. In addition it must somehow carry the information used by the host cell in synthesizing specific viral coat proteins. How these two processes are accomplished is suggested by the structure of DNA.

Structure of DNA

A structure of DNA that has great interest and significance to biology was recently proposed by Watson and Crick (37). On the basis of evidence from conventional chemical methods of analysis, electron microscopy, X-ray diffraction techniques of investigating molecular structure, and the model building approach, these workers suggest that DNA occurs in the form of long double right-handed helices in each unit of which two polynucleotide chains are wound around a common axis in such a way that their sugar-phosphate backbones form uniform helices with the purine and pyrimidine bases directed inward (Fig. 1).

The two chains are hydrogen bonded together through their purine and pyrimidine bases. These lie in planes to which the longitudinal axis of

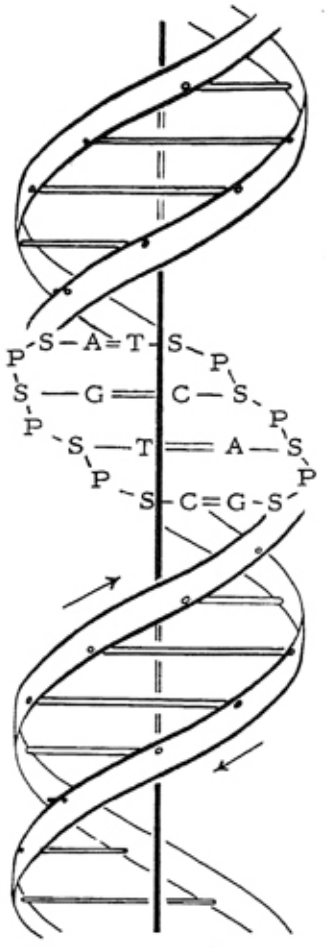


Fig. 1—Diagrammatic representation of the Watson-Crick DNA structure. P, phosphate; S, sugar; A, adenine; T, thymine; G, guanine; C, cytosine. Horizontal parallel lines symbolize hydrogen bonding between complementary bases. From Watson and Crick (37) with modifications.

the helix is perpendicular. A most significant feature is that structural considerations dictate that the base pairs consist of the purine adenine (A) hydrogen bonded to the pyrimidine thymine (T) or the purine guanine (G) similarly bonded to the pyrimidine cytosine (C). This means that the two chains have complementary base sequences. If a segment of one is A - T - G - C the paired one must have the sequence T - A - C - G to form the sequence of paired bases

A T G C
T A C G

where hydrogen bonds are represented by dots and the sugar phosphate backbones are indicated by dashes.

Direction is given to the polynucleotide chains by the orientation of the sugar units. The carbon atoms by which these are linked through oxygen

to the phosphorous atoms of the chain are numbered 3 and 5 as one progresses along the chain in one direction. In this sense the two parallel chains run in opposite directions.

The diameter of the Watson-Crick double helix is 20 Å. Its pitch is 34 Å and the distance along the axis between base pairs is 3.4 Å. There are thus ten base pairs per turn of the helix.

DNA Specificity

In the structure just described, specificity is presumed to be determined by the sequence of base pairs. Assuming no restrictions on the proportions in which the four bases occur in a single chain, it is clear that the number of different DNA structures that are possible will be 4^n where n is the number of nucleotides in the chain. Since the DNA in T2 and T4 bacterial viruses is equivalent to about 200,000 base pairs it is obvious that, even with considerable restriction on the proportions in which the four bases occur in a given chain, the opportunity for variability in the DNA of such a virus is practically unlimited.

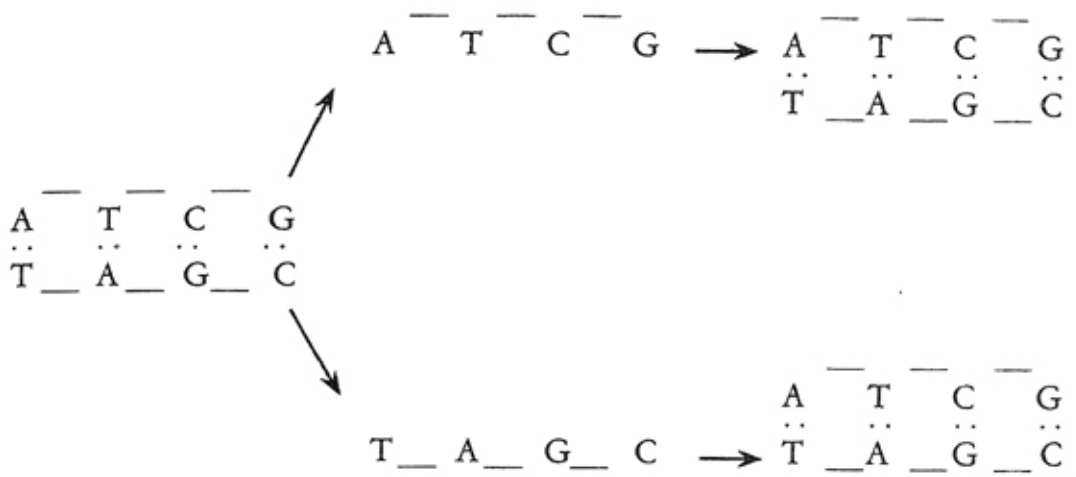
It is clear that in such a structure the specificity of one polynucleotide chain will be the complement of the other and that the double helix will carry no more information than either component chain alone. The stituation is not unlike a Morse code and its complement in which dots are replaced by dashes and dashes by dots.

If the essential genetic material of a bacterial virus is DNA and the Watson-Crick structure is correct, genetic specificity in this form must reside in the base sequence of its DNA.

Replication of DNA

One of the respects in which the Watson-Crick structure of DNA is so satisfying from a biological standpoint is that it is the only molecular model so far proposed that provides a reasonable basis for understanding how in principle a complex and highly specific molecule can be systematically replicated from an appropriate array of building blocks. On the basis of this model it is assumed that during the replication process the complementary polynucleotide chains separate by breakage of hydrogen bonds and that the single chains then acquire partners by selecting at each base level building blocks carrying the appropriate complementary bases. How the process occurs in detail and in just what form the building blocks are collected from the cell environment in which the replication occurs is not yet known.

In symbolic form the process can be indicated as follows:



It is not yet understood how the partners of the double helix separate at the time of reproduction—whether by untwisting (37), by systematic breakage and reunion (7), or in some other way. The problem is not a simple one, for molecular weight determinations of DNA indicate that the helices must have a length of at least 1000 turns. In the multiplication of a bacterial virus DNA equivalent to some 20,000 turns of the helix must duplicate once every minute and a half. At the end of this period each of the 100 or so descendant sets of DNA must be packed inside a virus head the diameter of which is about one thousandth the total length of the DNA, that is its quota.

The DNA Model and Spontaneous Mutation

Watson and Crick (37) suggests a mechanism by which mistakes could occur in the replication of a DNA unit. In its most stable, and hence most probable, tautomeric form an adenine base in the DNA helix forms hydrogen bonds with thymine. But a less probable tautomer will form such bonds with cytosine rather than with thymine. It is therefore suggested that if adenine is in the less probable state at the exact moment its complement is selected, cytosine will replace thymine in the daughter chain of a double helix and in the next replication a cytosine-guanine base pair will replace an adenine-thymine pair. In a similar way other substitutions might be made.

If this is the mechanism by which spontaneous mutations occur, the probability of an error in copying one base pair in a single replication process must be exceedingly small. Considering that a unit of DNA corresponding to a gene in the functional sense probably contains at least hundreds of base pairs and that spontaneous mutations for particular genes may occur with a frequency of less than 10^{-6} per generation, the chance of error per base pair per replication must often be two or three orders of magnitude less.

Presumably there are also other ways in which mutational changes can occur—by loss of base pairs, inversion of sequents, etc.

DNA and Gene Function

If, as the virus evidence suggests, DNA is the primary genetic material, then obviously the information carried by it must somehow be used in the process of gene function. It seems a good guess that this is accomplished by a transfer of specific information to macromolecules of other kinds. From various lines of evidence it seems reasonable that this transfer involves ribonucleic acid (RNA) as a first step.

Unfortunately no structure of RNA has yet been proposed that seems as plausible on either chemical or biological grounds as the Watson-Crick DNA double helix. There has therefore been little progress made in attempts to understand how the transfer of specificity of DNA to RNA is made.

A second likely step in gene function is the utilization of the information contained in RNA for protein synthesis. Again knowledge of the details of the process is meagre. An attractive possibility is that segments of RNA serve as templates on which specific proteins are constructed (11).

On the basis of these suggestions, functional genetic units would be those segments of DNA that, through RNA, correspond to specific proteins. Depending on the relation of number of nucleotides in such a unit to the number of amino acids in the corresponding protein, a functional gene might perhaps consist of a few hundred to a thousand or so base pairs.

Such an identification of DNA segments with functional genetic units or genes seems plausible enough for bacterial viruses where the experimental evidence indicates that during one stage of the life cycle, DNA is stripped bare of other material. But how about the stituation in higher forms? In bacteria, too, the case for the essential genetic material being DNA seems good from both the transforming principle evidence already summarized and the facts about transduction that will be presented later. 1

In the chromosomes of higher plants and animals protein is found associated with DNA in all phases of the cell division cycle at which chromosomes are easily investigated. During these stages the chromosomes are of an entirely different order of magnitude in cross sectional area from the DNA helices of bacterial viruses. While it is tempting to believe that the primary genetic material in these organisms is also DNA, the evidence for this is not as direct. Also one cannot be at all sure that the mechanism of genetic recombination of linked genetic material in the DNA of virus is basically the same as that between homologous chromosomes at meiosis in higher forms. Again the temptation to look for a single explanation is great. It is conceivable that in the so-called resting nuclei of cellular forms the genetic material may be reduced to the state of bare DNA.

What about the primary genetic information in those viruses that appear to contain RNA but no DNA—tobacco mosaic virus, for example? Presumably RNA is capable of carrying such information. If so, is RNA capable of direct replication or is it multiplied only indirectly via host cell DNA manufactured in response to viral RNA? Until more is known about the structure and function of RNA there seems little point in speculating further about questions of this kind.

Fine Structure of the Gene

Two recent lines of evidence promise to add greatly to our knowledge of the interrelations of gene function, mutation and recombination. Both are extensions of the approach so strongly advocated by Stadler—intensive study of individual genes.

The first involves an investigation by Benzer (4) of a series of mutant types of T4 virus of the colon bacillus. The members of this group of mutants are related in two ways. First, each of them differs from the original "wild type" virus by change in a single segment of their genetic material that is only about four percent of the total genetic map. Secondly, all members of the series are phenotypically alike. The members of the group are designated *rII* mutants.

To appreciate the evidence, it is necessary to understand the relation of wild type and *rII* mutant strains of virus to three strains of host cells designated B, K and S. On Petri dish cultures of all these hosts wild type virus produces characteristic normal plaques (circular clear areas in which bacterial cells have been lysed). On B cells, mutant viruses produce larger plaques with sharper margins than those of wild type viruses, whereas on S hosts, they produce normal plaques. On K hosts *rII* mutant viruses result in very few or no plaques at all; infected K cells are killed but usually release few or no infective viruses.

Because *rII* mutants grow normally in S hosts but produce no plaques (except by back mutation) on K hosts, it is simple technically to detect low frequencies of wild type recombinants produced by "crossing" two different *rII* mutants in an S host and subsequently plating on a K host. Recombinant viruses that are wild type will produce plaques whereas non-recombinants or double mutant recombinants will not. Therefore map distance is simply measured as twice the percentage of total virus particles (assayed on B or S hosts) that are capable of forming plaques on K host cells.

When many mutants of the *rII* group—distinguished from *r* mutants of other groups by their behavior on the three hosts mentioned—are studied carefully, it is found that they show variability in three respects, viz., (1) map position, (2) transfer coefficient (number of K cells that liberate virus—with some mutants of the *rII* group a few K cells do liberate a few viruses but in general plaques are not produced on K plates), and (3) reversion

index (proportion of reversions to wild type viruses per generation), which varies from 1 to 17,000 x 10^{-8}.

The more than 50 *rII* mutants studied in these respects are located at various places along a segment of genetic material about 8 map units long, the total map distance represented by all genetic material being estimated to be about 200. Within this region they fall into subgroups or microclusters. Recombination values as low as 0.013 map units have been measured with such subgroups.

A test essentially like the phenotypic test for allelism in higher forms can be made by infecting K hosts with two *rII* mutants of independent origin in such a way that most individual cells will be simultaneously invaded by both types. By the interactions observed in this way the *rII* genetic material can be divided into two contiguous segments, one about five units long and one about three. If two mutants located in a single segment on the basis of recombination data, simultaneously infect a given K cell, no lysis results. But if the two mutants lie in separate segments, lysis does result.

This mutual interaction is comparable to that observed in the double heterozygote involving two non-allelic recessive genes of a higher organism. Evidence that the *rII* mutants behave as recessives in such double infections is shown by the fact that a K cell so infected with wild type and *rII* mutant viruses releases both kinds of virus on lysis.

The evidence suggests two functional units in the *rII* cluster, corresponding to the two segments. Not only are the two units separable from each other by recombination but it is necessary to assume that mutant changes at different levels within one functional unit are capable of showing recombination. The shortest distance measured between two separable units is about one-twenty thousandth of the estimated total genetic material. Since there are about 20,000 turns of DNA helix in a virus of this type this distance presumably corresponds to about one turn of ten nucleotides. The limit of resolution of the method used by Benzer has almost surely not been reached. Conceivably in favorable cases it may be capable of measuring the genetic distance between adjacent nucleotides.

The functional genetic unit as determined by recombination data and the interaction test for allelism is about 4 map units long or about 4000 nucleotides. If all such functional units averaged this length, the total DNA would provide for 50 of them in a bacterial virus—assuming all DNA to be genetically active.

A significant feature about the *rII* mutants of Benzer is that they do not all behave in recombination studies as point lesions with the functional unit. Instead some suppress recombination over a considerable fraction of such a unit. It seems likely that these may represent what could be called intragenic structural rearrangements if the gene is defined as a functional unit.

They might well be micro-deficiencies or inversions. Such micro-rearrangements are, it will be recalled, just what Stadler predicted. And the evidence for them comes from the kind of study he so strongly advocated—intensive study by all available methods of specific genetic units.

Transduction in Salmonella

A second new approach to a genetic study of the internal structure of functional genetic units is based on the phenomenon of transduction as worked out in *Salmonella,* a relative of the colon bacillus, by Zinder and Lederberg (38).

In transduction, genetic material is transferred from one bacterial strain to another by means of a temperate virus, that is, a virus capable of reproducing in a provirus stage (as DNA) synchronously with its bacterial host and without apparent damage to the host. Occasionally such carried viruses spontaneously begin rapid multiplication, produce complete infective progeny, and lyse the host. Such "induction" of latent virus can also be brought about by certain experimental treatments such as ultraviolet irradiation.

If such a temperate virus is grown in a host of a given genetic type, recovered following lysis, and introduced into a host of another genetic type, it is found that one in ten thousand or so of the recipient bacteria will be changed genetically to correspond to the donor. If the two bacteria differ in two or more genetic traits usually only one is transferred at a time.

It is assumed that the transferring virus often carries, along with its own DNA, small segments of genetic material, presumably also DNA, of the donor cell. On establishment of the virus in the new host this carried genetic material is introduced into the recipient bacterium where it may replace homologous genetic material of the recipient.

The transduction phenomenon provides a sensitive test for determining whether bacterial mutants of suspected identity are located in identical positions in the genetic material. If they are, transductions between them will not be possible. But if they are located in separate places, one will be able to transduce the other with a frequency that decreases from an easily measurable value to zero as they approach each other in position.

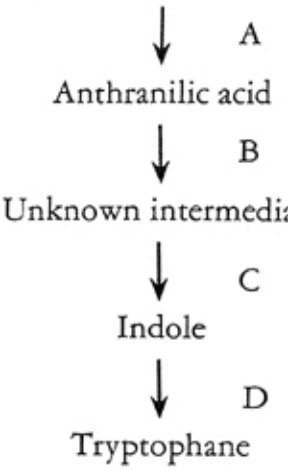
Demerec and coworkers (8) have made extensive use of this method in investigating the fine structure of genes of Salmonella. Since the evidence indicates the foreign segment of genetic material is incorporated by a process like crossing over, the method is in some respects like that used by Benzer in his studies of *rII* mutants of T4 virus.

First a large number of biosynthetically defective mutants were obtained. These were categorized according to the system of biosynthesis affected. Thus at one stage in the study there were 40 strains unable to synthesize cysteine, 11 that required tryptophane, plus nine other categories.

Systematic tests were then made for transductions within categories. The cysteine-requiring mutants fell into four groups. Transductions between members of different groups were frequent, but between members of a single group they were either much lower in frequency or did not occur at all.

With the tryptophane-requiring mutants four groups were identified by the same method.

The synthesis of tryptophane is known to involve the following steps:



All mutants of group A were found to be blocked prior to anthranilic acid. Those of group B were found to be blocked between anthranilic and the unknown intermediate. In the same way all group C mutants involved the next step and all D mutants the final step.

Linkage Detection by Transduction

If a strain of Salmonella carrying two mutants is transduced with virus grown in a wild type host, the frequency of double transductions will be low —approximately the value expected by coincidence of independent events—if the two mutants are genetically independent or loosely linked. But if they are closely linked the incidence of double transduction increases presumably because a single segment transferred from the donor can readily replace both mutants. Such evidence indicates that the transferred segments are small on the average. In addition to its use in detecting close linkage, the frequency of double transductions provides a method of determining the closeness of such linkage. Using donor and recipient strains differing from each other in three non-identical closely linked units, the order of these units can be inferred. This is true whether the units under study belong to a single group or to two or three.

In this way it has been determined that the three functional units (groups of mutants) tryptophane A, cysteine B and tryptophane D, are

linked in that order. One, eleven, and six independently obtained members of these three groups have been reported.

The Salmonella transduction work of the Demerec group indicates that in this bacterium there are many functional units corresponding to those of Neurospora and other organisms. Many of these are evidently concerned with the production of specific enzymes and, through them, with particular chemical reactions. These groups clearly correspond to what have been called genes in other organisms.

But, as in the case of Benzer's *rII* mutants in bacterial virus T2, these functional units are capable of being resolved into finer units by the transduction method. Thus in the seven cysteine mutants of group A, transduction tests for allelism show that there are five units capable of giving transduction in all combinations of two. A sixth mutant type gives no transduction with any other member of the group. These results indicate that the functional unit can be modified at any one of many levels and that from two such units modified at different levels a normal unit can be reconstructed. Some modifications (e.g., the cysteine mutant that gives no transduction with any other member of its group) involve a large fraction of the functional unit. Cases of this kind probably correspond to the Benzer *rII* mutants that show no recombination with other mutants in the same group. And like the *rII* mutants, these may well represent intragenic rearrangements such as deficiencies or inversions.

Transduction studies promise to contribute much more than they already have to our knowledge of gene structure and gene function. For example, there would seem to be no reason why there cannot be found in Salmonella an enzyme favorable for experimental study that is controlled by a functional unit capable of resolution by the transduction method. In this way investigation of the gene-enzyme relation can be extended to a comparison of fine-structure differences in both.

Conclusion

What, then, is a gene?

A consideration of available evidence suggests that the primary genetic material of many organisms is desoxyribonucleic acid. The polynucleotide chains of this material seem to consist of successive segments of perhaps several hundred nucleotides, each segment constituting a functional unit that serves to direct the specificity of a macromolecule such as a protein. In viruses and bacteria, evidence from recombination studies indicates that modifications can occur at different levels within such a unit. It is possible that the so-called pseudoalleles of higher forms represent a similar situation.

The question of whether non-specific material—for example, the calcium-magnesium bridges that Mazia assumes to hold segments of chro-

matin together —separates one functional unit from another cannot be given a final answer at present.

Evidence from studies of *rII* mutants of the T2 bacterial virus and of transduction, hints that the minimum unit of recombination, structural rearrangement, and mutation may be a single nucleotide. How then do we define a gene? If it were necessary to answer the question without additional information, the choice would have to be an arbitrary one. Possibly the time is approaching when additional terms will have to be coined to designate genetic units of different types. In the meantime it is important to make perfectly clear, as Stadler did, just what one has in mind in a particular case.

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The Gene

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THE central problem of biology is the physical nature of living substance. It is this that gives drive and zest to the study of the gene, for the investigation of the behavior of genic substance seems at present our most direct approach to this problem.

Current knowledge of the behavior of living cells presents two striking pictures. The first is the almost incredibly delicate balance of chemical reactions occurring in the living cell, by which energy is made available and by which the syntheses proceed that provide the materials for growth. The second is the behavior of the genic substance, which apparently guides these reactions. It is carried in the chromosomes in fine strands, which together make up only a minute portion of the substance of the cell. These strands are differentiated along their length into hundreds of segments of distinctive action, and, therefore, presumably of distinctive constitution, which we speak of as the genes. The genic substance is reduplicated in each cell generation. Its distinctive segments, in many known cases, determine whether or not a specific chemical reaction will occur, presumably, in some cases at least, by determining the production of a specific catalyst.

The great bulk of the substance of the cell apparently consists of materials produced by the afore-

Dr. Stadler, before his death on 12 May, asked that this paper be sent to Science. *It is the valediction, and a remarkable one, of a great geneticist.*—EDITORS.

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mentioned guided reactions. The nature and behavior of these materials, so far as we know them, do not require the assumption that they have properties essentially different from those of nonliving matter.

The genic substance, on the contrary, appears to have properties quite different from those with which we are familiar from our knowledge of the physical science of nonliving matter. Modern physical science gives us no model to explain the reduplication of the gene-string in each cell generation, or to explain the production of effective quantities of specific enzymes or other agents by specific genes. The precise pairing and interchange of segments by homologous gene-strings at meiosis also suggest novel physical properties of this form of matter. These facts indicate that a knowledge of the nature and properties of the genic substance might give clues to the distinctive physical mechanisms of life.

The difficulties in the study of the genic substance are obvious. It cannot be isolated for chemical analysis or pure culture. The possibility of direct analysis of specific segments or individual genes is, of course, even more remote. The properties of the genes may be inferred only from the results of their action.

Furthermore, a critical study of the effects of a single gene may be made only by comparing individuals wholly comparable in genotype except for a difference in the one gene concerned. This means that gene mutations are essential for such comparisons, since it is only by gene mutation that we can identify individuals differing only by the effects of a single gene. The prospect of determining the properties of the gene is, therefore, dependent upon the development of valid methods for the study of gene mutation.

It is appropriate to cite here the monumental contributions of H. J. Muller to the investigation of this problem. More than 30 years ago he recognized clearly the unique significance of gene mutation in the study of the physical nature of life (*1*) and boldly attacked the imposing technical problems that blocked its experimental investigation.

The difficulties of analysis that have been mentioned are not different in kind from those involved in other problems in which the properties of hypothetical elements must be inferred from their effects—for example, in the problems of molecular or atomic structure. In such studies, the investigator proceeds by constructing the simplest model that will fit the known facts and then attempting to apply every significant experimental test of the predictions that may be made from the model. By a series of successive approximations, the model finally evolves to a form that seems to provide the most plausible mechanism for the behavior observed. The study of the physical nature of the gene from purely genetic evidence is closely comparable to this.

These difficulties of analysis are mitigated in some degree by the possibility of parallel investigation of certain problems of mutation through direct observation of the chromosomes. Although the gene-string itself is below the limit of microscopic visibility, its behavior is such that it provides a visible shadow, so to speak, in the chromosome. Some alterations of the gene-strings are readily detectable by visible alteration of the chromosomes. The cytogenetic analysis of individual mutations provides a wholesome check on hypotheses derived from the statistics of mutation frequencies.

An illuminating example of this is afforded by certain interpretations of the evidence on mutation rate as affected by x-ray treatment and by temperature. At an early stage in the study of x-ray-induced mutations, Delbrueck (*2*) constructed a tentative "atomic physics model" of the gene, as inferred from the frequency of point mutations observed under varying physical conditions. This has become widely known through its application and discussion in the engaging little book *What Is Life?* (*3*), published several years later by the eminent theoretical physicist, Erwin Schrodinger.

In this view, the gene is considered a molecule, and the observed mutations are considered to represent its transitions from one stable state to another, as a re-

sult of thermal agitation or the absorption of radiant energy. The linear-dosage curve and the constancy of mutation yield, regardless of variation in the time factor, show that the x-ray-induced mutations result from single "hits"; the constant proportionality of mutation yields to ionization, regardless of variation in wavelength, shows that the unit "hit" is an ionization. Calculation of the volume within which these hits must occur to account for the mutations observed provides a basis for estimating the average size of the gene-molecules postulated. This turns out to be of the order of 1000 atoms. The relative frequency of spontaneous mutations at different temperatures permits the calculation of the activation energy required for the occurrence of a mutation, which turns out to be about 1.5 ev. Unstable genes are assumed to have correspondingly lower activation energies, and the fact that temperature affects their mutation rate less than that of normally stable genes is in agreement with expectation on this basis. The energy spent in one ionization is about 30 ev, and it is therefore to be expected that irradiation will cause the mutation of any of the genes, regardless of their relative stability under normal conditions. The proportional increase in mutation rate will, therefore, be much less for genes distinctly unstable at ordinary temperatures than for genes of normal stability. These expectations also are realized.

This is an impressive picture, but it has been evident for many years that it has no valid relationship to the experimental data from which it was derived. The detailed analysis of individual cases among the x-ray-induced mutations has shown clearly that many of these result not from a structural change in a gene but from some alteration external to the gene, such as physical loss or rearrangement of a segment of the gene-string. We have no basis for estimating the proportion of such extragenic mutations among the total of mutations observed and no ground for assuming that this proportion is the same among the mutations observed under the various experimental treatments.

The basis of the model is the assumption that the

statistics of observed mutation are in fact the statistics of structural alteration of the molecules that constitute the gene-string. The investigations of specific mutations contradict this assumption and show that the model has no basis in reality.

It is interesting to reflect that if the determiners of heredity had chanced to be of a lower order of magnitude, below the level at which the experimental study of individual cases is possible, we might still be constructing more and more refined models of the gene on this pattern. As the predictions made from the model were contradicted by experimental results, we would change the various numerical values, or introduce additional variables, or perhaps, if necessary, even create additional hypothetical units. But the model would remain essentially an imaginary construct inferred from mere numbers of mutations, for we would have no possibility of contradicting the plausible assumption that one mutation is as good as another.

What Is a Gene?

The early studies of gene mutation were concerned mainly with problems of technique arising from the extreme rarity of the phenomenon. Although the mutations of *Oenothera*, on which the mutation theory was based, had proved illusory, it soon became evident that mutant alterations do occur that are inherited as if they were due to changes in individual genes. The comprehensive genetic analysis of *Drosophila* by Morgan and his coworkers showed numerous cases of this sort—in fact, almost all the loci shown on the gene-map represented the mutant occurrence of visible alterations which, on subsequent tests, proved to be inherited in typical Mendelian fashion. These were assumed to be due, in each case, to a change of the wild-type gene to an alternative form, producing a recognizably different phenotypic effect. The frequency of these mutations, however, seemed far too low to permit experimental investigation of the conditions affecting their occurrence.

Muller (*4*) pointed out in 1917 that gene mutations

resulting in inviability ("lethals") are probably more frequent than mutations permitting survival with modified phenotype ("visibles"). In experiments extending over the next 10 years (*5*), he developed various special techniques by which it was possible to determine the total number of lethal mutations for all loci within a given chromosome or region. These total frequencies proved to be high enough to permit significant experimental comparison of mutation frequencies under different temperatures. The loci yielding lethal mutations were distributed over the chromosomes approximately as expected from the distribution of loci for visible mutants, and it was concluded that the lethal mutations might legitimately be used as an index of gene mutations in general.

Meanwhile, many attempts to increase the frequency of genetic alterations by external treatments had been made, including studies with various chemical, radiological, and serological treatments, and studies in which various plant and animal forms were used. None of these experiments gave conclusive proof of an effect of any experimental treatment on the frequency of mutation, although in several of the experiments there were genetic alterations that may have been induced by the treatment. The failure of proof was due to two difficulties: (i) that of proving that the genetic alterations observed in the progeny of treated individuals were in fact due to the treatment rather than to some genetic irregularity present in the treated strains, and (ii) that of showing statistically convincing increases in the frequency of mutations in the treated group. What was needed was a genetic technique suitable for the detection of mutations in adequate numbers in an organism in which the gene-determined inheritance of the mutant characters could be readily demonstrated.

The "ClB" technique with *Drosophila*, designed by Muller, was admirably suited to this purpose, and x-ray experiments with this technique (*6, 7*) demonstrated beyond question a very strong effect of x-rays on the frequency of mutation. The total frequency of lethals in the X-chromosome was increased more than

100-fold. In addition, many visible mutations were found, including dominants as well as recessives, and including mutants previously known from their spontaneous occurrence as well as many mutants not previously observed.

These experiments were promptly followed by others designed to test more critically the genic nature of the induced mutations. The mutant lethals might be suspected of being deficiencies; even the visibles could conceivably be due to short deficiency or gene destruction. But if the treatment could induce mutation to a variant allele and could, in further applications, induce reverse mutation to the parental allele, it was argued, the two mutations could not both be due to gene loss. Induced mutation and induced reverse mutation at the same locus were shown to occur in a number of loci of *Drosophila* in experiments by Patterson and Muller (*8*) and by Timoféeff-Ressovsky (*9*).

Subsequent experiments with a wide variety of forms among the higher plants and animals and with microorganisms showed the broad generality of the effects of ionizing radiations upon the frequency of mutation. In later experiments, ultraviolet radiation and various chemical treatments were also shown to affect mutation frequency.

The analysis of the induced mutations, however, soon indicated that the accepted definitions and criterions related to genes and gene mutations needed reconsideration.

The purpose of experiments with gene mutation is to study the evolution of new gene forms. The techniques for studying gene mutation are, therefore, designed to measure the frequency of these changes in the genes. But a change in the gene may be recognized only by its effects, and it soon became clear that various extragenic alterations might produce the effects considered characteristic of typical gene mutation (*10*).

Thus the working definition of mutation necessarily differs from the ideal definition. It is this working definition that must be considered in generalizing

from the experimental evidence. The mutations experimentally identified as gene mutations may include not only variations due to alterations within the gene but also variations due to losses of genes, to additions of genes, and to changes in the spatial relationships of genes to one another. To identify these mechanical alterations, certain tests were applicable. But there was no test to identify mutations due to a change within the gene; it was simply inferred that the mutants that could not be identified as the result of specific mechanical causes were, in fact, due to gene mutation in the ideal sense (*11*).

When we conclude from an experiment that new genes have been evolved by the action of x-rays, we are not simply stating the results of the experiment. We are, in the single statement, combining two distinct steps: (i) stating the observed results of the experiment, and (ii) interpreting the mutations as due to a specific mechanism. It is essential that these two steps be kept separate, because the first step represents a permanent addition to the known body of fact, whereas the second step represents only an inference that may later be modified or contradicted by additional facts. When the two steps are unconsciously combined, we risk confusing what we know with what we only think we know.

The widely held belief that the frequency of gene mutation may be greatly accelerated by x-ray treatment was an illusion of this kind. Its basis was the use of the term *gene mutation* with two distinctly different meanings. Gene mutation was thought of as a change in the constitution of a unit of the genetic material, producing a new gene with altered gene action. Gene mutation was identified in experiments by the occurrence of a mutant character inherited as if it were due to a change in a gene.

The mischief involved in the use of the same term for the two concepts is obvious. To insist that x-rays induce gene mutation because the mutants induced satisfy all the accepted criterions of gene mutation, and that these mutants represent qualitative changes

in specific genes because that is what we mean by gene mutation, is to adopt the dictum of Humpty Dumpty in *Through the Looking-Glass.* "When I use a word," Humpty Dumpty said, "it means just what I choose it to mean—neither more nor less."

Now our concept of the gene is entirely dependent upon the occurrence of gene mutations. If there were no gene mutations, we could not identify individual genes, because the total genetic effect of a single chromosome would be inherited as a unit. If the mutations we interpret as gene mutations are in fact due to alterations affecting groups of genes, then the entities that we will recognize as genes will be in fact the corresponding groups of genes. The significant ambiguity is not in our definition of gene mutation but in our definition of the gene itself, because any definition of gene mutation presupposes a definition of the gene.

The discussion of these difficulties and of the possibility of remedying them by more rigorous definition of experimental concepts is only an application to biology of the operational viewpoint that has become commonplace in modern physics, largely as a result of the critical studies of P. W. Bridgman (*12*). As Bridgman notes, this sort of critical reconsideration, made necessary in physics by the development of relativity, is essential in scientific thinking if the methods are to be made elastic enough to deal with any sort of facts that may develop. The essential feature of the operational viewpoint is that an object or phenomenon under experimental investigation cannot usefully be defined in terms of assumed properties beyond experimental determination but rather must be defined in terms of the actual operations that may be applied in dealing with it. The principle is not a new one; it has been recognized, at least implicitly, in the work of individual scientists from an early period. William James stated it essentially in his lectures on pragmatism (*13*), illustrating it with a quotation from Wilhelm Ostwald:

> Chemists have long wrangled over the inner con-

stitution of certain bodies called tautomerous. Their properties seemed equally consistent with the notion that an instable hydrogen atom oscillates inside of them, or that they are instable mixtures of two bodies. Controversy raged but never was decided. "It would never have begun," says Ostwald, "if the combatants had asked themselves what particular experimental fact could have been made different by one or the other view being correct. For it would then have appeared that no difference of fact could possibly ensue; and the quarrel was as unreal as if, theorizing in primitive times about the raising of dough by yeast, one party should have invoked a 'brownie' while another insisted on an 'elf' as the true cause of the phenomenon."

What is a gene in operational terms? In other words, how can we define the gene in such a way as to separate established fact from inference and interpretation? The definition may take into account not merely the evidence from experiments on the occurrence of mutations but also the evidence from experiments on the inheritance of genetic differences of any kind, or from any other experiments that bear on the nature of the gene. The definition may specify attributes of the gene that can be determined by recognized experimental operations, whether these are attributes already established in past experiments or attributes that might be determined in future experiments.

Operationally, the gene can be defined only as the smallest segment of the gene-string that can be shown to be consistently associated with the occurrence of a specific genetic effect. It cannot be defined as a single molecule, because we have no experimental operations that can be applied in actual cases to determine whether or not a given gene is a single molecule. It cannot be defined as an indivisible unit, because, although our definition provides that we will recognize as separate genes any determiners actually separated by crossing over or translocation, there is no experimental operation that can prove that further separation is impossible. For similar reasons, it cannot be defined as the unit of reproduction or the unit of

action of the gene-string, nor can it be shown to be delimited from neighboring genes by definite boundaries.

This does not mean that questions concerning the undetermined properties mentioned are meaningless questions. On the contrary, they are the all-important questions that we hope ultimately to answer by the interpretation of the experimental evidence and by the development of new experimental operations. The operational definition merely represents the properties of the actual gene, so far as they may be established from experimental evidence by present methods. The inferences from this evidence provide a tentative model of the hypothetical gene, a model that will be somewhat different in the minds of different students of the problem and will be further modified in the light of further investigation.

The term *gene* as used in current genetic literature means sometimes the operational gene and sometimes the hypothetical gene, and sometimes, it must be confessed, a curious conglomeration of the two. The resulting confusion may be strikingly illustrated in seemingly contradictory statements by two such gifted and clear-sighted geneticists as Richard Goldschmidt and A. H. Sturtevant. Goldschmidt, after reviewing the evidence on position effect, states that genes do not exist (*14*), or at any rate that the classical theory of the corpuscular gene must be discarded (*15*). Sturtevant, citing the evidence that chromosomes are regionally differentiated, that particular regions are necessary for particular reactions in the organism, and that these particular regions behave as units in crossing over, states "These propositions . . . prove the existence of genes" (*16*).

Goldschmidt is essentially correct if, by the gene, we mean the hypothetical gene, and the particular hypothetical gene that he has in mind. His positive conclusion that the gene does not exist is prone to misinterpretation but apparently means only that this hypothetical gene does not exist. His contention that the properties commonly ascribed to "the classical,

corpuscular gene" go far beyond the evidence is, I think, fully justified.

Sturtevant is correct if, by the gene, we mean the gene of the operational definition, since this implies no unproved properties. If it were true that there are no discrete units in the gene-string, Sturtevant points out, the most direct way of establishing the fact experimentally would still be by studying the properties and interrelationships of these distinguishable regions. These are the genes of the operational definition.

What is the operational definition of gene mutation? We have recognized that our studies of gene mutation have significance for the major problem only to the extent that they identify and analyze the mutations that represent the evolution of new hereditary units. But it is obvious that no operational definition of gene mutation in this sense can now be formulated—for these hereditary units are not the genes of the operational definition; they are the hypothetical genes postulated in our interpretation of the experimental evidence. To say that no operational definition is now possible is only to repeat in different words the foregoing statement that we have no positive criterion to identify mutations caused by a change within the gene, and that the alterations interpreted as gene mutations in experiments are merely the unclassified residue that cannot be proved to be due to other causes. The major objective in further investigations must be to develop such criterions.

Study of the Mutation of Specific Genes

The main purpose of this paper (*17*) is to emphasize the unpleasant fact that significant progress in our understanding of gene mutation requires the investigation of the mutation of specific genes. The fact is unpleasant because the various technical difficulties that arise from the very low frequency characteristic of mutation are at their worst when the study must be made on single genes, particularly on the spontaneous mutation of single genes. The unpleasant statement is a fact because, as we have seen, it is hopeless

to identify and exclude the spurious or extragenic mutations in experiments on mutation rates at miscellaneous unspecified loci.

The chief advantage in focusing the study on the single gene is that this makes it possible to substitute the direct experimental analysis of specific mutants for the application of generalizations assumed to apply to mutations at all loci. Each mutant studied may add to the background of detailed information available for the diagnosis of other mutants of the same gene.

An important further advantage is that the specific loci selected for study may be loci with unusual technical advantages for the recognition and analysis of their mutants. For example, the genes R^r and A^b in maize, like other known genes in various species, yield spontaneous mutants that are clearly distinct from the forms produced by recognizable short deficiencies at these loci. This does not prove that the spontaneous mutants are not due to still smaller deficiencies, but it supplies a convenient screen for identifying a large class of deficiencies without further investigation. Another very useful aid in discriminating between gene loss and gene alteration is available for the recessive allele *a*. This allele, although phenotypically distinguishable only by the loss of *A* action, may be distinguished from gene deficiency by its response to the mutagenic gene Dotted (*Dt*), in the presence of which it reverts sporadically to the dominant allele *A*. The retention of the *Dt* response provides a criterion to exclude gene loss in the interpretation of experiments on spontaneous and induced mutation of *A*. A technical advantage of a different sort is provided by the *R* alleles. The phenotypic effect of *R* is such that a large number of alleles may be objectively distinguished by very slight differences of plant color intensity and pattern. A gene with equally variable allelic forms, if identified only by its effect on some all-or-none response, would seem to have only two alleles, and its mutations would not be detectable except for those that crossed the line between these two

distinguishable levels of action. Another advantage of great practical importance is that both *R* and *A* are genes affecting endosperm characters and are, therefore, suitable for the identification of mutations in large populations. Both are apparently genes of such trivial effect physiologically that their mutants survive with no detectable loss of viability.

The effective analysis of the diverse genetic phenomena that may result in the origin of a Mendelizing variation may not be impossible in intensive studies of the mutations of suitable selected genes, despite the fact that it seems hopeless in studies of mutation at miscellaneous, unspecified loci.

These considerations are of no account if the frequency of spontaneous mutation of the single gene is actually too low to permit effective experimental study. We cannot safely avoid this difficulty by selecting for study the genes of unusually high mutation frequency, because there is no assurance that the mechanism responsible for the behavior of "unstable genes" is representative of the mechanisms concerned in typical gene mutation. The use of microorganisms that permit effective screening for mutants in virtually unlimited populations would remove the difficulty, but unfortunately these do not provide the critical genetic background essential to the study.

A technique for determining the spontaneous frequency of mutation of specific genes is practicable in maize for mutation rates ranging as low as about one per 1 million gametes (*18*). A test of eight genes, unselected except for the technical advantage of showing their effects in the endosperm, yielded mutations in all but one of the genes tested, the mutation frequencies ranging from about one to about 500 per 1 million gametes tested (*19*). The genes that yielded mutations in sufficient numbers to permit the comparisons showed rather wide variation in mutation frequency in different cultures. The gene *R*, for example, yielded no mutations in large populations in some cultures, but its mutation rate in other cultures ranged as high as 0.2 percent. Later studies have shown

that such differences are due in part to differences intrinsic to the *R* allele concerned and in part to differences caused by factors modifying the mutation rate of R (*20*). Such factors are apparently quite common, since a study in which only strong effects could be detected indicated the occurrence of such modifiers in three of the seven regions marked (*21*).

The average mutation rates determined are rather low for effective experimental investigation of factors affecting the mutation rate and even for the extraction of adequate samples of mutants for individual study. However, the fact that mutation rates are so readily affected by diverse modifiers makes it feasible to extract strains in which the mutations of specific genes may be made frequent enough to permit direct experimenal study.

Detection of Spurious Gene Mutations

The development of criterions for identifying gene mutations of evolutionary significance is difficult even in the study of selected genes of the most favorable properties. In past studies, the problem has been given a disarmingly simple appearance by various assumptions, some of which were unwarranted, and some of which have been invalidated by later discoveries.

For example, we tend to feel that some of the mutations detected in our experiments must be qualitative changes in the genes concerned, for surely qualitatively altered genes have arisen in the course of evolution. This is mainly responsible for the widespread belief that, even though some of the apparent gene mutations identified are demonstrably false, "true" gene mutations must be included in the unclassified residue.

This belief is fallacious. Granting that qualitatively changed genes must have been evolved by mutation at rates high enough to permit experimental investigation, there is no assurance that the steps in their evolution are represented in the mutants that are found in our mutation experiments. When we set out to identify mutants in a mutation experiment, we must con-

fine ourselves to mutations of relatively large effect, large enough to set the mutant beyond the range of varying expression due to environmental and genetic modifiers. If mutant changes occur within the narrower range, we have no way of identifying them. There is no good evidence against the occurrence of such subliminal mutations. The assumption of the high constancy of the gene is backed by evidence only concerning the rarity of the distinct mutations. If convincing evidence were adduced tomorrow to show that genotypes breed true only as a statistical result of sampling in each generation in populations of genes genetically fluctuating over an imperceptible range, there is nothing in our present knowledge that would contradict this conclusion.

A study of *R* alleles of diverse origin showed the common occurrence of minute differences in the level of plant-color expression (*22*). Such allelic differences would not be expected if the only source of variation in this gene were mutation of the type that we study in our experiments, but they would be expected as a result of subliminal mutation.

If subliminal mutations occur, it is possible that this type of mutation accounts largely or wholly for the evolution of new gene forms in nature. Thus it is quite possible that the sharply distinct mutations identified in our experiments may be exclusively the result of extragenic phenomena.

A second assumption, or group of assumptions, is concerned with the possibility of distinguishing gene mutation from gene loss. It was originally supposed that induced recessive "visibles" could safely be considered gene mutations, on the assumption that all genes were essential to survival. This was contradicted by various instances of cytologically demonstrable deficiencies viable in haploid tissue or in hemizygous individuals, or viable as homozygotes in diploid individuals. Such cases were relatively few, but since both the cytological and the genetic criterions of deficiency approach the limit of their range of effective application as the deficient segment becomes smaller, there is

reason to suspect that physical loss may be responsible for observed mutations also in cases in which deficiency cannot be demonstrated. As we have become better acquainted with individual genes and their functions, the assumption that genes, as a rule, are individually essential to life has lost its plausibility.

Mutation to an intermediate allele is sometimes considered evidence against loss mutation. This involves another assumption, that of the unitary nature of the gene—an assumption made consciously and with careful consideration in the early development of gene theory, but one that must be seriously questioned in the light of later evidence. It is only on the hypothesis that multiple alleles are variant forms of a single unit that we may exclude the possibility of their occurrence by loss mutation. On the hypothesis that they represent different mutations in a complex of closely linked genes, we could account for mutation to different levels by the loss of different segments of the chain.

The basis for the choice of the unitary hypothesis is perhaps best shown in the considerations underlying the classical criterion of allelism. These were stated by Morgan in 1919 (*23*) as follows:

> Probably the most important evidence bearing on the nature of the genes is that derived from multiple allelomorphs. Now that proof has been furnished that the phenomena connected with these cases are not due to nests of closely linked genes, we can probably appeal to these as crucial cases. . . . The demonstration that multiple allelomorphs are modifications of the same locus in the chromosome, rather than cases of closely linked genes, can come only where their origin is known. . . .

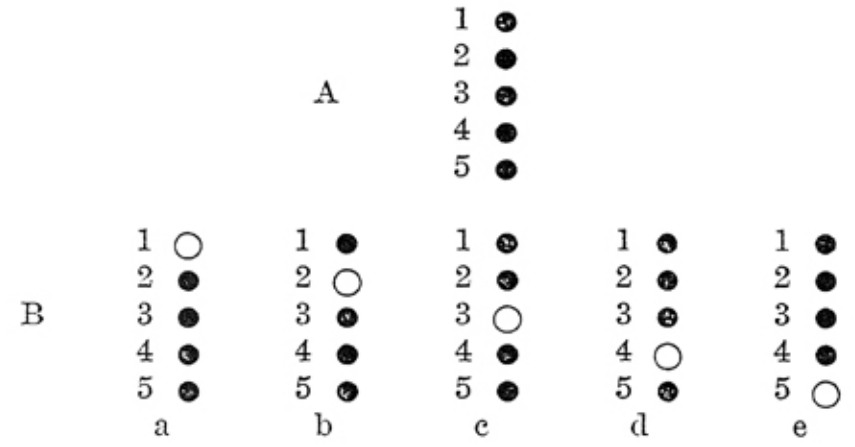
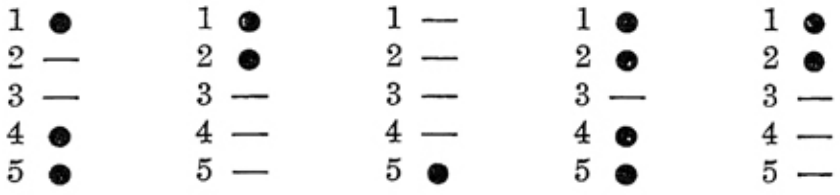


Fig. 108 [in part]. Diagram illustrating mutation in a nest of genes so closely linked that no crossing over takes place.

> Let the five circles of Fig. 108, *A* represent a *nest* of closely linked genes. If a recessive mutation occurs in the first one (line *B*, *a*) and another in the second gene (line *B*, *b*), the two mutants *a* and *b* if crossed should give the atavistic type, since *a* brings in the normal allelomorph (*B*) of *b*, and *b* that (*A*) of *a*. . . . Now this is exactly what does *not* take place when members of an allelomorphic series are crossed —they do not give the wild type, but one of the other mutant types or an intermediate character. Evidently independent mutation in a nest of linked normal genes will not explain the results if the new genes arise directly each from a different normal allelomorph.

It will be noted that the test rules out the existence of the nest of closely linked genes only on the assumption that each mutation must be an alteration of a single number of the group. If, instead, each mutation were a loss of one or more contiguous numbers of the group, the fact that crosses between them might commonly show them to be allelic would not rule out the "compound gene" as the basis of the multiple allelic series. This is illustrated in the following diagrammatic arrangement:



The "compound gene" is in a sense a contradiction in terms, for the hypothetical gene is unitary by definition. But the genes identified in our experiments cannot be made unitary by definition. The five genic elements represented in the diagram are not actually parts of one gene; they are five genes. But if certain multiple allelic series have a basis of this type, it would be possible to establish the fact experimentally only in the cases most favorable for analysis. Accordingly, there might be many cases in which the segment of the gene-string identified experimentally as a single gene might actually be a cluster of genes of identical or similar effect.

The notion of the compound gene, or some equivalent unit, may prove to have significance, since there may be special relationships among the clustered elements that mark them off as a group from adjoining unrelated elements. One of these may be interrelationships in gene action between the clustered elements, which could lead to the occurrence of position effects when members of the cluster are separated by crossing over or translocation. This may be a basic factor in the explanation of position effect in general. Another relationship to be expected is synaptic equivalence, leading to the opportunity of unequal crossing over. It is the latter that concerns us here.

A striking example of minute deficiencies simulating gene mutations is provided by the "crossover-mutants" of R^r. Certain R^r alleles consist of at least two independently mutating genic elements: (P), determining anthocyanin pigmentation of certain plant tissues and of the pericarp, and (S), determining anthocyanin pigmentation of the endosperm and embryo. The crossover-mutants R^g and r^r result from unequal crossing over and must, therefore, involve the

loss of (P) in the one case and of (S) in the other. They give no cytological or genetic indication of deficiency, and they are wholly normal in development in the haploid gametophyte, as is shown even by the very sensitive test of competitive pollen-tube growth in the transmission of the mutant through male germ cells. The crossover-mutants are wholly indistinguishable in appearance and genetic behavior from the noncrossover mutants occurring in the same cultures.

The occurrence of unequal crossing over within the *R* complex yields some interesting indications of the genetic nature of multiple allelic series and of the possible role of gene losses in relation to seemingly qualitative mutations. In addition to (P) and (S), there are other phenotypically recognizable genic elements of the *R* complex. In certain R^r alleles of dilute pigmentation, both plant and seed color are dependent upon a single genic element (D). In various R^r alleles of unusually strong pigmentation, there appear to be additional elements determining certain aspects of plant-color expression. In addition, there are various distinguishable aleurone-color types such as "Stippled," "Marbled," "Navajo-spot," and so forth, some occurring with plant color and some without. Each of the distinguishable complexes may be regarded as one of a long series of multiple alleles of the gene *R*.

Let us pause a moment to clear the terminology. To avoid confusion I shall refer to the recognized alleles of *R* under their customary italicized designations (R^r, R^g, r^r, R^{Nj}, and so forth), although the analysis shows that several of these so-called "alleles" are actually complexes of two or more genes.

The term *genic element* will be used for any gene-like constituent identified as a component of one of the *R* alleles. The use of this term does not, in the absence of further evidence, necessarily imply that the element is unitary. The genic elements are designated by symbols not italicized, such as P, S, D, and so forth.

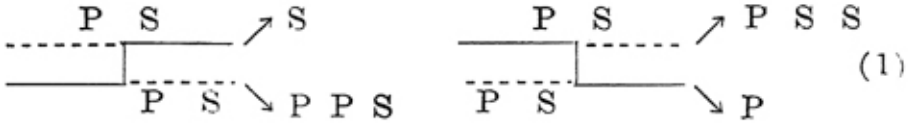
In addition to the crossover mutants there are numerous noncrossover mutants. A noncrossover r^r mutant is presumably of constitution "P s" rather than

merely "P." The postulated element (s) is a "null" element phenotypically but presumably would function synaptically in the same way as "S." These postulated elements are designated "s," "p," "d," and so forth.

The complex may, of course, include other null elements from past mutations in which the parental elements are unknown. These as a class are designated as "n."

In several instances noncrossover mutants to intermediate levels of seed-color expression occurred including various dilution and pattern types. These are designated "S^d," "S^s," and so forth.

Once any two of these genic elements have become established in neighboring positions in the same chromosome, an opportunity is provided for unequal crossing over, which may ultimately lead to the development of more complex gene clusters. For example, the aforementioned crossover mutants resulted from interchanges as follows:



The crossover-product "S" was recognizable as a crossover mutant R^g and the crossover-product "P" as crossover mutant r^r. The crossover-products "P P S" and "P S S" were not recognizable, but these represented the production of potential new alleles carrying three genic elements instead of two. By using distinguishable forms of S or P in the original compound, the addition-crossovers may be made recognizable, and by this means it is possible to produce such new synthetic alleles as *R* (Stippled-Navajo), and so forth. In this manner, it would be expected that more complex clusters would develop by successive steps, unless the gene is one whose action sets a closer limit on the viability of its duplications.

The great variety of genotypes that might be expected to represent possible members of the allelic

series may be illustrated by a few examples as follows:

1)	S	S	p	n	
2)	S	P	P	n	S
3)	D				
4)	D	S	P		
5)	S^s	P	D		

Alleles (2) and (4) would be of the standard R^r type, (3) would be of the dilute R^r type, (1) would be of the R^g type, and (5) would be a spotted aleurone type with plant color. In general, the differences between the alleles are due to extragenic, rather than intragenic, alterations, but this is not necessarily true of the phenotypic difference between (4) and (5).

With regard to the relationships between the genic elements of the complex, the concepts of allelism and locus have little meaning. All members of the complex are homologous with one another; presumably all have arisen through a long series of mutations from some single ancestral gene. In a sense, all may be considered allelic to one another. For example, the question "Is S^n (the seed-color element in R^{Nj}) allele to S?" has no significance, because there is no way in which S^n can be shown to have any different relationship to S than to P or to any other element of the complex. The same is true of such a question as "Is the element (D) proximal or distal to (P)?" It may be proximal in one stock and distal in another; in a stock in which it is proximal, a short series of unequal crossovers will suffice to move it to a distal position.

Although different alleles may have widely different numbers of genic elements, none is actually a deficiency. In terms of the postulated origin of the cluster, all of those with more than a single element may be considered duplications. On the other hand, when we arbitrarily take as the standard type an allele carrying several genic elements, other alleles with fewer elements will appear as deficiencies, and the mechanisms that produce them as mutants from the standard type will be mechanisms of gene loss.

The same mechanisms proceeding in the case of a gene-complex whose separable elements are identical in action might produce only a linear series of multiple alleles showing various grades of dilution, or they might produce no multiple series of alleles at all.

The increasing number of cases in which clustering of genes of identical or similar effect is proved or indicated (*24–27* and others, *28* and *29* for references) suggests that unequal crossing over may be a significant factor in the production of seemingly qualitative allelic differences.

Another simplifying assumption was that mutant changes in gene effect must represent some transformation of the gene itself rather than some alteration affecting its expression. It was this assumption that made the demonstration of x-ray-induced mutation and reversion of the same gene seem critical proof of the induction of intragenic alterations. The assumption was definitely contradicted by the evidence of position effect. This evidence showed conclusively that a mutation did not necessarily represent a transformation or loss of the gene concerned; instead, it could be the result of a translocation affecting the expression of the unchanged gene.

The remarkable studies of McClintock (*30, 31*) on mutational behavior in maize, as affected by the introduction of a chromosome-9 undergoing the breakage-fusion-bridge cycle, have shown the far-reaching importance of this limitation in the experimental study of gene mutation. In the presence of this structurally unstable chromosome, many of the type genes present, including genes in chromosome-9 and genes in other chromosomes, show mutation to unstable recessive forms characterized by various types of chromosomal irregularity. The study of the unstable mutants and their reversion leaves little doubt that the phenomenon is due to some reversible inhibition of the expression of the genes concerned.

In some cases the mutations are accompanied by detectable chromosomal aberrations at or near the locus showing instability, but in other cases no cyto-

logically detectable chromosomal alteration is associated with the occurrence of the mutation. In many cases the instability of the recessive mutant and the occurrence of the associated chromosomal irregularities are dependent upon the presence of a complementary factor designated "activator" (Ac), and when this factor is removed the mutant behaves as a stable recessive with normal chromosomal behavior.

McClintock has also shown that the control of reverse mutation of the recessive *a* by *Dt* (Dotted) may be a reaction of the activator type. In the presence of the aberrant chromosome-9 and in the absence of *Dt*, the standard *a* allele has given occasional endosperm dots apparently due to mutation to *A*. This strongly indicates that the standard *a* is a repressed *A*, and, if so, its reversion under the influence of *Dt* must also be due to some modification of conditions affecting gene expression.

Whether or not there is acceptance of the hypothesis that these manifestations of unstable gene behavior are brought about by the transposition of invisible bits of heterochromatin to the locus of the gene affected, this brilliant investigation clearly shows that expression effects may be the actual cause of apparent gene mutations, even when the mutation observed shows no indication of a change of position or of any associated chromosomal alteration.

The resulting difficulty in the analysis of observed mutations further emphasizes the necessity for carrying on the analysis with the advantages of the detailed study of mutation at specific loci. If we think of these results in terms of the generalizing assumptions characteristic of the study of mutation *en masse,* we may be inclined to apply the findings to the nature of gene instability in general, or even to the nature of mutant alleles in general. If we think of them against the background of diverse mutations of some intensively studied gene, we are inclined to make detailed comparisons of the mutants of this category with those of other types and other modes of origin in the hope of developing criterions that distinguish mutants of dif-

ferent kinds.

Meanwhile, in the study of gene mutation, we are for the present in an anomalous position. A mutant may meet every test of gene mutation, and yet, if it is not capable of reverse mutation there is ground for the suspicion that it may be due to gene loss, while, if it is capable of reverse mutation, there is ground for the suspicion that it may be due to an expression-effect. The only escape from this dilemma is through the more intensive study of the mutations of specific genes selected as best suited to detailed genetic analysis, in the hope of developing more sensitive criterions for the identification of gene mutations.

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