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 aforementioned 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.

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.

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 Schrödinger.

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 result 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 saitsfy 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 constitution 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 investiga-

tion, 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 confine 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. . . .

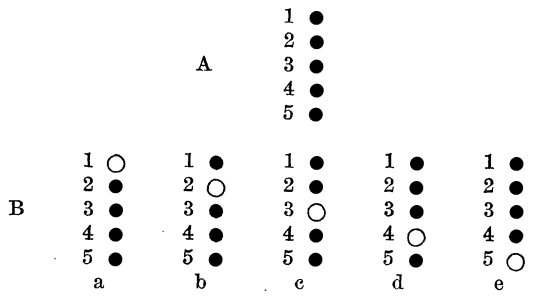


Fig. 108 [in part]. Diagram illustrating mutation in a nest of genes so closely linked that no crossing over takes place.

> 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:

1 ●	1 ●	1 —	1 ●	1 ●
2 —	2 ●	2 —	2 ●	2 ●
3 —	3 —	3 —	3 —	3 —
4 ●	4 —	4 —	4 ●	4 —
5 ●	5 —	5 ●	5 ●	5 —

The "compound gene" is in a sense a contradiction in terms, for the hypothetical gene is unitary by defi-

nition. 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 (*24*). 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.

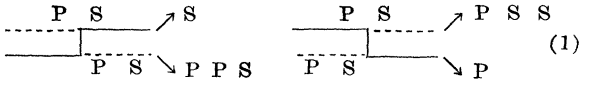
The term *genic element* will be used for any gene-like constituent identified as a component of one of the *R* alleles. The use of this term does not, in the absence of further evidence, necessarily imply that the element is unitary. The genic elements are designated by symbols not italicized, such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



(1)

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^{Ni}) 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 cytologically 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 my 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 different 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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Erosion Phenomena

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THERE are a number of interesting erosion phenomena that are not the result of an equilibrium between heat exchange by radiation and by convection, yet the effects are in some cases so similar to such equilibrium effects that at first glance these erosion phenomena appear to belong in the same class.

Fluted rocks. In many mountain regions one finds very striking erosion phenomena in limestone: well-formed channels leading downhill. Their bottom is invariably rounded, and the ridges between the channels are exceedingly sharp. These channels vary in width from a fraction of an inch to several feet, and their length may easily attain 30 ft. On the side walls of the larger channels new, smaller channels are formed. They too lead in the direction of maximum inclination. They are undoubtedly formed by rain water containing, of course, carbon dioxide.

The explanation is a very old and simple one: If a slightly inclined rock surface, probably originally polished by glacier action, is not perfectly flat, then after each rain the deeper places will remain wet longer than the protruding parts. At these places the erosion proceeds faster than at elevated, drier regions. The differences between high and low are, therefore, accentuated by rain water. The edges between two channels get more and more elevated above the deeper parts of the rock and, after each rain, they are the first to dry. These ridges between the channels get sharper and sharper, and they can, without exaggeration, be compared to knife edges. At some places the water seems to have found a vertical crack, and these cracks are then widened to deep crevasses, which may have a width of several feet. It is well known in such mountain regions that sheep can be killed when they fall into these holes.

Similar formations can be observed in gypsum rocks. I have climbed, with the aid of a rope, down into some of these vertical shafts, which had a perfectly circular cross section and the walls of which were quite smooth. These "chimneys" in gypsum rocks are harder to explain, but they may well be related to the better known fluted rocks in limestone.

Action of acid on files. It is well known that if a dull file is dipped for a few minutes into concentrated hydrochloric acid, it will come out considerably sharper than it was before the dipping. This phenomenon is very similar to the formation of fluted rocks, but it is more difficult to explain because there is no reason why the action of the acid should be less strong on the ridges than on the grooves. Here we apparently need a geometric explanation.

Let us assume that we start with an iron plate that is fluted with alternating convex and concave cylinder surfaces, all of the same radius of curvature. If the acid acts with the same speed on the ridges and on the grooves, the radius of curvature of the grooves will increase until the concave cylindrical surfaces of the grooves meet, whereas the radius of curvature of the ridges will decrease. When these radii have reached a zero value, the grooves meet and a maximum sharpness of the ridges is attained. From then on any further