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MUTATION

Early studies on variation were concerned with phenomena that were in part due to recombination, and the origin of new types could be studied effectively only when the question could be formulated in terms of the origin of new genes. The first question was: Do new genes in fact arise, or is all genetic variability due to recombination of preexisting genes? This question was seriously discussed—though the alternative to mutation seems to be an initial divine creation of all existing genes.

It is true that most strains studied are of hybrid derivation—or may be so—and that recessives may remain undetected for many generations. The result is that, while one may argue that the origin of new genes is a logical necessity, it is not so easy to specify that a given gene must be of recent origin. Perhaps the clearest evidence in the early studies came from the sweet pea, which was known to be descended entirely from a single small collection of wild material (from Sicily) and to have been propagated for many generations, mostly by self-pollination, before new types appeared. The history of the various color types was known, and left no doubt that particular genes had arisen suddenly in cultivated material. But most of the newly arisen forms in such cases are recessive to the original type, and, on the long-accepted "presence and absence" hypothesis, represent merely losses of genes—not the acquisition of new ones. As late as 1914, Bateson discussed the possibility that evolution had come about solely through the loss of genes, followed by recombination.

In diploid organisms there are only two classes of genes whose mutability can be studied simply and effectively—newly arising dominants, and sex-linked genes, where in the heterozygous sex each individual is effectively haploid. Both were used in early work, and special methods were developed fairly quickly for the study of autosomal recessives, especially in self-fertilizing plants.

In the 1890's de Vries studied the genetic behavior of variegation in the flowers of Antirrhinum, the snapdragon. Here the flowers are white

or yellow, with red stripes, the variegation being recessive to the self red condition. On a variegated plant, the size of the red areas is variable, and sometimes includes an entire branch. De Vries showed (1901, *Die Muta-tionstheorie)* that the flowers on these red branches behave like the red flowers on a wholly red F_1, that is, on self-pollination they give 3 red : 1 variegated. The results were not given a Mendelian interpretation be-cause, at the time the experiments were done, de Vries did not know about Mendelism. He did, however, suggest that some kind of segrega-tion *(Spaltung)* was occurring, so that self color was somehow split off from the variegated element; he knew, and emphasized the point, that no true whites were produced.

Correns (1909, 1910) studied similar cases in the four o'clock, Mirabilis, the most detailed account concerning variegated leaves. He gave a Mendelian interpretation but thought of segregation rather than of mutation—though, like de Vries, he found that the change was in one direction only: from variegated (pale green and dark green) to self dark green. His main emphasis was on the change in somatic tissues from a homozygous variegated condition to a heterozygous (self/variegated) one. Neither de Vries nor Correns seems to have been clear that the variegation itself, within individual flowers or leaves, could most easily be interpreted as being due to the occurrence, late in development, of the same change responsible for large sectors, which could then be transmit-ted. This view, and the interpretation of the whole series of events as re-current gene mutations, was developed by R. A. Emerson (1914) as a result of his studies on the variegated pericarp of the "calico" type of maize. He also pointed out what was evident in his results and in those of de Vries and of Correns (when finally mutation was thought to cause variegation), namely, that the mutation regularly occurred in only one of the two genes in a single homozygous cell.

The regular and frequent occurrence of mutations in such cases was made clear by these papers, but there was, and still remains, a reluctance to generalize from them about the mutation process, since the usual fre-quency is so very much less than they report—and is for that reason much more difficult to study.

The early work on Drosophila, especially that of Bridges, furnished several instances of the occurrence of new dominant genes, and many new sex-linked recessives, in pedigreed material where the event could be analyzed in some detail. These examples confirmed the conclusion that mutation occurs in a single gene in a single cell, and that it can occur at any stage of development. In fact, Muller concluded that the frequency

per cell is probably the same for each stage in the germ line.

These results were merely qualitative, since the frequencies were too low for a quantitative study and were also strongly influenced by the personal equation of the observer. What was needed was an objective index, and one that would recognize a class of mutations that was frequent enough to give significant numerical values. Both of these requirements were met in the system devised by Muller for the study of newly arisen sex-linked lethals in Drosophila.

As first used by Muller and Altenburg (1919), the method depended on the study of the sex-ratios from individual females that were heterozygous for sex-linked "marker" genes. This technique made possible an objective and unambiguous determination of the frequency of sex-linked lethals, but it was very laborious, since rather detailed counts had to be made for each tested chromosome. There was a suggestion of an increase in the rate of occurrence of new lethals with an increase in temperature; this was later confirmed with improved techniques. It was also evident that the rate per unit time could not be as high in man as in Drosophila, for the approximate 1 : 1 human sex-ratio would then be quite impossible.

Muller then improved the technique by using the "ClB" chromosome that he found in his experiments. This is an X chromosome that carries a crossover reducer (later shown to be an inversion), a lethal, and the dominant mutant gene Bar. As is well known now, this chromosome made it possible to detect new sex-linked lethals without anesthetizing the flies or making counts—merely by rapid examination of individual culture bottles. In this way one could test many more chromosomes than possible ever before and thus could obtain adequate data on lethal frequencies.

The first great advance through the use of this technique was the demonstration of the mutagenic action of X rays (Muller, 1927).

X rays were reported in 1895 by Roentgen, and, in the next year, radiation burns caused by them were reported by L. G. Stevens. There followed a rapidly increasing volume of literature on the biological effects of X rays and (beginning in 1900) of radium rays. "Radiation sickness," described in 1897 by Seguy and Quenisset, and by Walsh, was characterized by heart symptoms, headaches, and insomnia. The first successful therapeutic use seems to have been by Steenbeck, who in 1900 reported the destruction of a small tumor in the nose of a patient. Radium burns were reported in this same year by Walkoff and by Griesel—a result confirmed in 1901 by Becquerel and Curie, who deliberately ex-

posed themselves. In 1901 Rollins reported that heavy doses to guinea pigs were lethal without the occurrence of any visible surface changes.

The carcinogenic properties were reported in 1902 by Frieben, who described a carcinoma of the skin following X-ray exposure. In 1903 Bohn concluded, as a result of studies on the gametes and the fertilized eggs of sea urchins, that the primary damage caused by radium treatment was in the chromatin. In 1904 Perthes reported on the cytological effects of radium on the developing eggs of Ascaris and suggested that the chromosomes were fragmented—though he wondered if this result might be due to accidental cutting by the microtome knife in making his preparations. In 1905 Koernicke treated Lilium with radium and concluded that there was a true fragmentation of the chromosomes.

Numerous attempts were made to induce mutations by high-energy radiations and also by other physical and chemical treatments. Mac-Dougall, Vail, and Shull (1907) treated Oenothera with radium, and Morgan (1911) and Loeb and Bancroft (1911) used it with Drosophila. In all these cases, a few mutants occurred, but at the time it seemed most probable that they were not produced by the treatment, since mutations also occurred in the controls. It is now clear that the genetic techniques then used were not adequate for the demonstration of an increase in mutation frequency of the magnitude likely to occur.

Genetic effects were found, however, in Drosophila by Mavor. In 1921 and 1922 he showed that X rays produce a marked increase in the frequency of nondisjunction, and in 1923 he found that they also affect the frequency of crossing over. Anderson confirmed the nondisjunction effect and in 1925 showed that one of the exceptional females produced had her two X's attached—this being the first induced chromosome rearrangement.

When Muller applied his "ClB" technique to the study of sperm treated with X rays, it was apparent that there was a marked increase in the frequency of newly arising lethals. This paper (1927) marks the first clear example of the artificial induction of mutations.

Stadler had begun his studies with barley at about the same time that Muller's work with X rays began, but since he was using an annual plant, his results were not available until after Muller's were published. His first 1928 paper did furnish independent confirmation of Muller's result, on different material studied with a different technique.

Stadler irradiated seeds of barley. At the time of treatment, the embryo was already formed, and each of the several main shoots of the developing plant was represented by a single diploid cell. When the plant

was mature, each shoot was separately self-pollinated and the seeds were planted. Any induced recessive segregated in the ratio 3 : 1 and could be identified as induced, since it would not appear from other shoots of the same plant. In all, Stadler found forty-eight mutations in distinct seedling characters from irradiated seeds, and none from a large control series. Some of these seeds had been given X rays, others had been exposed to radium—the latter proving to be also mutagenic.

These results of Muller and of Stadler at last opened the way to an experimental attack on problems having to do with mutation, and they and others began an extensive study of these problems—a study that is still being very actively prosecuted.

Muller, in his first account, concluded that high-energy radiation is dangerous not only to the exposed individual, but to his descendants as well. This conclusion has come to be generally understood and accepted, and is now of much public concern in connection with the medical and dental use of X rays, and the worldwide distribution of radioactive isotopes produced by nuclear fission.

In this same paper, Muller made two tentative suggestions that were soon shown, by Muller himself and by others, to be incorrect: first, that "spontaneous" mutations might be largely or wholly due to normal background radiation, and second, that the relation between dosage and mutation rate might be exponential rather than linear.

Stadler, in his second paper in 1928, reported (in an abstract about half a page long) three major discoveries made with barley:

1. Seeds soaked in water to initiate germination gave almost eight times the mutation rate of dormant seeds.

2. Mutation rate was independent of the temperature at the time of irradiation.

3. The relation of mutation rate to total dosage was linear—doubling the dose doubled the mutation rate.

In 1929, Hanson and Heys studied the induction of lethals in Drosophila by radium. They interposed lead shields of different thicknesses and recorded the ionization (using an ionization chamber) in each treatment. The curves for ionization and for mutation rate were superposable, leading to the conclusion that ionization is responsible for the mutations and that the relation is a simple, direct one—a "one-hit" phenomenon. In 1930, Oliver confirmed this conclusion by showing that varying the dose by varying the duration of exposure to a constant X ray source also gave a linear curve relating dosage to mutation rate. It was apparent that projecting this curve to a zero dosage did not give zero mutations, and Mul-

ler and Mott-Smith showed in this same year that the amount of background radiation is far less than would be required to produce the normal "spontaneous" frequency of mutations.

It came to be very generally accepted that total ionization is all that need be considered in connection with radiation-induced mutations, at least within a strain. Stadler's dormancy results should have suggested that it was dangerous to argue from sperm (which are essentially dormant) to actively growing tissues; but it was not until rather recently that it was shown (by Lüning, Russell, and others) that there are differences in dose response in different tissues and developmental stages in Drosophila and in mice. These studies, now being actively prosecuted, fall outside the scope of this book.

The induction of mutations by chemical means was attempted by many people over a long period, but until 1941 there were no clear and convincing positive results. In that year Auerbach and Robson obtained clear evidence that mustard gas is mutagenic. They were led to study it because pathologists had found much similarity between the appearance and behavior of skin burns induced by mustard gas and by irradiation. The mutation effect was observed in a (British) government-supported war project, and the information was classified and could not be published until 1946. With its publication began the present active and productive study of chemical mutagens—another development outside the scope of this book.