Perspectives

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Seventy Years Ago: Mutation Becomes Experimental

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IN 1927 H. J. MULLER (1890–1967) published in *Science* a paper entitled "Artificial Transmutation of the Gene." It reported the first experimental production of mutations and opened a new era in genetics. The title is curious. Why *transmutation* rather than *mutation*? The answer emerges from another paper (MULLER 1928a), written in 1926. After reviewing the repeated failure of efforts by many workers to modify the mutation rate, MULLER asked the question: "Do the preceding results mean, then, that mutation is unique among biological processes in being itself outside the reach of modification or control,—that it occupies a position similar to that till recently characteristic of atomic transmutation in physical science, in being purely spontaneous, 'from within,' and not subject to influences commonly dealt with? Must it be beyond the range of our scientific tools?" MULLER thought of his radiation experiments as parallel to those of RUTHERFORD, only a few years earlier, demonstrating experimental transmutation of chemical elements. Like the physicists, who were attracting a great deal of public attention at the time, MULLER had tampered with a fundamental natural process and had succeeded in mastering it. He was an instant celebrity.

The 1927 paper is also curious in another way, for it presented no data–no dosage measurements, no numbers, no statistical analysis. MULLER simply reported qualitative results and rough comparisons, *e.g.*, a mutation-rate increase of "fifteen thousand percent." But a paper without data invited skepticism, and the skeptics included no less than T. H. MORGAN, who was always suspicious of speculations and invariably asked for the data.

MULLER's idea was clearly to establish priority. He noted that many of the mutations were repeats of those found earlier. Most were recessive, but a few were dominant. Many were lethal or sterilizing, and there were dominant lethals, not easy to detect. In addition to gene mutations, MULLER reported a number of chromosome rearrangements, especially translocations. He suggested mutation as a cause of cancer. And in this, his first paper on the subject, he began his lifetime crusade against indiscriminate use of high-energy radiation, a crusade bolstered by the later demonstration that mutation was linearly related to dose, down to doses as low as could be practically studied.

In the summer of 1927 MULLER gave a major paper at the Fifth International Congress of Genetics in Berlin. Typically, he scribbled the paper in transit and was still preparing slides up to the time of its presentation. The talk is said to have been confusing, but the message was clear. This time he gave the full details and the skeptics were silenced. Helpful as he was throughout his life, CURT STERN got the paper typed, and it was published the next year (MULLER 1928b).

Biologists generally accepted mutation as the ultimate basis of evolution. Furthermore, mutation promised a way to get at the nature of the gene. Yet mutations were so rare that there was only anecdotal information. In a few months MULLER found more mutant genes than the total from all Drosophila labs up to that time. His discovery was independently confirmed by L. J. STADLER, who started experiments with barley and other plants at about the same time (ROMAN 1988; STADLER 1997). The slower life cycle in these plants meant that his results appeared somewhat later, but he clearly deserves recognition along with MULLER, although he didn't always receive it. Other geneticists immediately jumped on the bandwagon, and the field of radiation genetics was on its way.

Although he was actively engaged in many aspects of Drosophila genetics and was an active contributor in various ways to the MORGAN group, MULLER's interest centered on mutation. His first experiments started in 1918, so his radiation paper represented the culmination of a decade of work. MULLER's two full reports, one written before the X-ray discovery (1928a) and the other immediately after (1928b), report a remarkable saga. The first paper occupies an entire issue of *Genetics*, 79 pages. In minute detail, he described the various experiments, each successive one being an improvement and coming closer to providing convincing quantitative data. His innovations and their updates are now standard Drosophila methodology.

The problem from the beginning was the rarity of mutations. Early on, MULLER decided that lethals were better material than visible mutations for quantitative study. For one thing, they were far more numerous. For another, the results were unambiguous; with rare exceptions there were no survivors (or only a few pitifully weak ones). The "personal equation" was eliminated; lethals could be identified as well by the average technician as by a sharp-eyed CALVIN BRIDGES. Soon MULLER began devising ways to identify all the lethal mutations on a chromosome, thereby multiplying the per-locus mutation rate by the number of lethal-producing loci on the chromosome.

Early in his work MULLER realized the value of "C factors," crossover suppressors, later proven to be inversions. He exploited these to construct balanced lethal systems in which lethal mutations, in addition to those necessary to keep the system balanced, could be accumulated over many generations. Then, by elaborate mating schemes, he could render the new mutations homozygous and locate their approximate position by linkage with known markers. This permitted further enrichment of the number of mutations by summing the mutations that had accumulated over many generations.

In these accumulation experiments, MULLER foreshadowed the work of MUKAI (1964), who employed this idea to measure the spontaneous rate of mutations with small effects on viability. MULLER had argued that mutations producing small, statistically detected effects on viability and fertility were the most numerous class, and MUKAI showed it.

MULLER's most famous Drosophila stock involved an *X* chromosome that he called *ClB*. *C* stands for a crossover suppressor, *l* is a recessive lethal, and *B* is the Bar "gene," later proven to be a duplication. Using the crosses that are now familiar to every student of elementary genetics, MULLER was able to measure the rate of lethal mutation on the *X* chromosome with almost no ambiguity. MULLER was constantly on the lookout for time-saving methods, and this was one of his best. Since an F_2 culture descended from a new recessive lethal contained no males, it was not necessary to examine the flies in detail, but only enough to ascertain whether males were present. This could be done by examining the flies in the vial with the naked eye or, a bit better, a hand lens sufficed. Figure 1 shows MULLER demonstrating his favorite low-tech instrument. The *ClB* method provides an easy way to map the location of new lethals, and MULLER quickly exploited this. He showed that lethal mutations are distributed over the entire *X* chromosome, roughly uniformly, although concentrated at the "left" end.

Another innovation was a "double *X* experiment," which made use of L. V. MORGAN's strain in which two *X* chromosomes were attached at the centromere. By crossing treated males to such females, any *X*-linked visible mutations were immediately apparent in males of the next generation.

From the beginning, MULLER thought that perhaps the best way to get at the question of whether the mutation rate was immutable was to study the effect of temperature. If mutation behaved like ordinary laboratory chemical reactions, the rate should double or triple with each rise of 10°. Therefore, throughout most of the several years of study in the early 1920s, temperature was varied, with increasingly precise results in the later experiments. Controlling temperature was no easy task in those days of poor equipment and poorly supported labs. MULLER did experiments in the MORGAN lab in New York, at Woods Hole in Massachusetts, at Rice Institute in Houston, and at the University of Texas in Austin. It was especially difficult to arrange cool temperatures in the hot Texas summers. He covered the cultures with a wet cloth on which an electric fan blew. Despite the crudity of the experimental conditions, he was able to compare two sets of experiments that, while each varied considerably, differed on the average by about 8°.

By showing that the mutation rate could be influenced, MULLER made mutation a researchable subject. In his words (1928a): "Perhaps the most hopeful feature of the present data is that they show that mutation is indeed capable of being influenced 'artificially'—that it does not stand as an unreachable god playing its pranks upon us from some impregnable citadel in the germ plasm." Later the temperature effect was given a theoretical interpretation (see SCHRÖDINGER 1944).

It is a short intellectual step from realization that the mutation process has a high temperature coefficient to thinking of radiation as a source of activating energy. Although MULLER justly receives great credit for radiation mutagenesis, the greater contribution is his development of techniques whereby mutation could be studied experimentally and measured reproducibly. In three monumental papers—actually only two, since the *Science* paper gave no details—MULLER provided essentially all the basic techniques used in the burst of radiation experiments done in the next several decades.

In those days, many geneticists thought that best way to get at the nature of the mysterious gene was through mutation. Perhaps the way the gene mutates could tell them what it is. Of course, genetic history has been quite different. The nature of the gene was discovered, not by the kinetics of mutation, but by the identification of DNA as the genetic material, by advances in the chemistry of large molecules, and by some clever model building by WATSON and CRICK. Now the tables are turned; knowledge of the gene is used to understand mutations and mutagens, not the other way around.

It is striking that for two decades after MULLER's discovery, no convincing evidence of chemical mutagenesis was presented. Although many chemicals were tried,

FIGURE 1.—H. J. MULLER and a student, DALE WAGONER, about 1961.

and some experiments would today be regarded as successes, none passed the extreme standards of rigor that the genetics community demanded. If there was an early dogma that mutations could not be induced, there was the new dogma that only radiation could do this. Chemical induction of mutations had to wait for MULLER's protégé, CHARLOTTE AUERBACH, to demonstrate the mutagenicity of mustard gas during World War II (BEALE 1993).

MULLER believed that radiation-induced mutations were essentially the same as spontaneous ones, while STADLER thought, correctly as it turned out, that they were mainly deletions (ROMAN 1988). MULLER hoped that he had discovered a way of inducing mutations important for evolution. Perhaps the hope fathered the belief, for he continued to hold to his original view, despite ever-increasing evidence to the contrary. It is unappealing to consider that we are deleted amoebas.

MULLER's LIFE

HERMAN JOSEPH MULLER, known to his intimates as Joe, was born in 1890 in New York City. His father must have been highly intelligent, for he had graduated number one in the intellectually elite and intensely competitive environment of City College of New York. The senior MULLER had hoped for a scholarly career in the field of international law. But the death of his own father had required that he take over the family business, making metal castings. By all reports he disliked the business, but reveled in his after-hours intellectual pursuits. He took Joe to museums and discussed Darwinism along with his social views. Alas, he died when the boy was nine years old. His busy mother continued to further his interest in nature.

MULLER was able to attend Columbia University, but only by working part time during the school year and in summer jobs. Yet he graduated with honors. His major subject was physiology, but he was attracted by MORGAN and his two brilliant students, A. H. STURTEVANT and C. B. BRIDGES. The excitement in the fly lab was such that it was inevitable for MULLER to gravitate to the group. But although he was associated with the fly group, he never was a real insider. It must have been a busy time for him. On a typical day he rode the subway to Cornell Medical School to teach in a physiology lab, then to Columbia for classes and a visit to the fly lab, then downtown where he taught an evening course in English for foreign students, and then back home by the subway.

The MORGAN lab was a single room, small enough for everyone to participate in the conversation. Everyone knew what everyone else was doing, and it must have been very hard to trace the history of an idea that was so thoroughly batted around. It was a heady environment and must have been intensely exciting for the participants. Drosophila genetics was advancing rapidly, and almost every experiment yielded something new. Yet the environment was not tranquil, for MULLER at least. MORGAN held that the origin of ideas was not important; the important thing was to do the experiment and get the data. Almost certainly MULLER, with his quick mind and creative imagination, contributed more than his share of ideas. And he had a sensitive ego. In any case he began to feel that he was not getting sufficient credit from MORGAN, and the rift persisted.

MULLER completed his thesis, on linkage and crossing over, in 1915 in his usual last-minute frenzy. He accepted a position at Rice Institute with his friend JULIAN HUXLEY. Summers were spent at Woods Hole. He returned to Columbia in 1918 and there began the serious study of mutation. His two-year appointment ended in 1920 and, to his great disappointment, was not extended. So he took a job at the University of Texas.

MULLER was recruited by J. T. PATTERSON, an embryologist, who among other things worked out the embryology of armadillo quadruplets. He provided MULLER with equipment, money, and a student assistant, a contrast to Columbia where he had had to pay for vials and assistance from his own not-too-deep pockets. Later, PATTERSON arranged to get MULLER an X-ray machine. Another Texas faculty member was T. S. PAINTER, a cytologist. Soon both PAINTER and PATTERSON were working with Drosophila.

MULLER worked extraordinarily hard, mainly on his mutation studies, but also on many other things. He discovered a pair of identical human twins that had been reared apart. He discussed the near-absence of polyploidy in animals, the failure of genes to function in spermatozoa, balanced lethals, distribution of crossovers, polyploid segregation, and dosage compensation among other things. He coined the useful words *hypomorph, hypermorph, antimorph,* etc. He formulated his ideas about what the gene had to do, the most remarkable property being to copy errors, that is, to mutate. He was fascinated by bacteriophage and this led to what must be MULLER's most famous statement, a remarkably prophetic one published in 1922:

> If these d'Herelle bodies were really genes, fundamentally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. They are filterable, to some extent isolable, handled in test tubes, and their properties, as shown by their effects on the bacteria, can then be studied after treatment. It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them. Hence we cannot categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all. Must we geneticists become bacteriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists? Let us hope so.

According to MULLER's later recollection, the previous speaker on that occasion thought this was a clever fantasy and congratulated MULLER on his sense of humor. Such farsightedness continued through his life; another often-cited example is his Pilgrim Trust Lecture, delivered in 1945 (LEDERBERG 1991).

Although his research brought him fame, MULLER was not satisfied at Texas. He felt, correctly, that PATTERSON and PAINTER were exploiting his ideas. Why wasn't he pleased, for he had many more ideas than he could personally test? At the same time he was involved in assorted leftist political activities and sponsored a radical student group. Two Russian geneticists worked in his laboratory. He became increasingly depressed by the racism and inequalities of wealth in the United States.

Germany seemed like a good place to further his socialist ideas, so in 1932 he joined the laboratory of TIMOFÉEFF-RESSOVSKY in Berlin. This was in the Brain Research Institute, headed by OSCAR VOGT. MULLER arrived only a few months before Hitler rose to power. VOGT refused to fire Jews, with the result that there were break-ins at the Institute and at VOGT's home, some of which MULLER witnessed. Staying in Germany became hopeless.

By his leftists activities, MULLER had burned his bridges in Texas, so he accepted a position at the Genetics Institute in Leningrad. The next few years were extremely productive. Salivary chromosomes had been discovered, and MULLER's Russian group became leaders in exploiting this powerful breakthrough. Yet this period of great productivity didn't last long. Genetics came under the diabolical influence of TROFIM LYSENKO, whose extravagant promises of higher crop yields caught Stalin's eye. LYSENKO's Lamarckian views became official and he became the dominant figure in Soviet genetics. The man who brought MULLER to Russia, N. I. VAVILOV, was later imprisoned and died (CROW 1993). So did the two Russians who had worked with MULLER in Texas.

More of MULLER's colleagues disappeared while others remained active "in inverse proportion to their honor." MULLER's plan for getting out of Russia was to take a leave of absence to work in the blood bank of the International Brigade in Spain in the fight against Franco. In this way, he would not seem disloyal to the Soviet regime and this could perhaps protect his surviving Russian friends. It didn't help much. Several perished or were imprisoned and others survived by doing nongenetic work.

After leaving Spain, MULLER had no luck finding a job in the West. Finally in 1937 he got a temporary position in WADDINGTON's Institute at the University of Edinburgh. Compared with the early situation in Russia, conditions were poor. He was without any help and had a hard time in the cold Edinburgh winters; he never was comfortable working with gloves as the others did. A notable event in this period was his supervising CHARLOTTE AUERBACH, who was interested in trying to discover chemical mutagens (BEALE 1993).

MULLER's chronic hard luck continued. Again he was in the wrong place, and when World War II began he tried to return to the United States. Again, no job. He finally obtained a temporary position at Amherst College, replacing HAROLD PLOUGH, who was on leave doing War work. Finally, in 1945, FRANK HANSON and WARREN WEAVER of the Rockefeller Foundation suggested MULLER to FERNANDUS PAYNE, head of the Zoology Department and Dean of the Graduate School at Indiana University. PAYNE was a Drosophila geneticist who had worked in the MORGAN lab. He couldn't care less about MULLER's leftist background, his reputation as a poor undergraduate teacher, or his "difficult" personality. He said that he already had several prima donnas on his staff and one more wouldn't matter. And he knew that MULLER was good with graduate students, and of course he was familiar with MULLER's great research.

So, at last in 1945, at age fifty-five, MULLER had a permanent position with stimulating colleagues, supplies, equipment, assistants, and graduate students. The Nobel Prize came a year later. Except for three periods on leave, in Hawaii, at City of Hope in California, and at the University of Wisconsin, MULLER remained at Indiana University for the rest of his life. He died in 1967.

MULLER's life was complicated and difficult, one disappointment after another. He was caught up in both of the two tragic dictatorships of the century, each with its way of perverting genetics. His stubborn idealism and strong personality often led to difficulties. His work was constantly being interrupted. Yet his work was his life, and usually it meant long days and seven-day weeks.

Clearly, MULLER was a complicated person. His great scientific intellect contrasted with his poor social skills. He could muster overwhelming arguments for a theory or for a political view, yet he did not realize that argumentative overkill is not always the way to win converts. He could be petty in personal relations, in contrast to his great idealism for mankind in the large. Even after becoming famous, he was excessively concerned that he receive credit for all his discoveries, no matter how minor. Yet, he could be charming, witty, and above all, a most stimulating conversationalist, be the subject genetics, society, or politics. And all his personal foibles recede in the glow of his great scientific achievements.

ELOF CARLSON (1981) has written an excellent full-length biography of MULLER, on which we have relied heavily. Some of our material is from CROW (1990). MULLER, himself, prepared a sampling of his papers (1962). Although it is impossible to get more than a small sample of work in a single volume, this is an excellent way to get an idea of the breadth, variety, and depth of MULLER's contributions. To quote JOSHUA LEDERBERG's Foreword, "Thoughtful reader—you will find a world of rediscovery here."

Several earlier *Perspectives* essays have touched on MULLER and his work. They include CROW (1988, 1995), GREEN (1996), LEDERBERG (1991), LEWIS (1995), PAUL (1988), ROMAN (1988), and STADLER (1997).

MULLER AS A PERSON

Each of us was well acquainted with MULLER, both being his intellectual descendants. One of us (S.A.) was MULLER's "son," that is, graduate student. The other (J.F.C.) was a "grandson," having been a student of one of MULLER's earliest students, W. S. STONE. We here record a few separate memories.

Some memories of a graduate student (S.A.): When I entered his laboratory in 1951, MULLER was still a dynamo of activity—working seven days a week, staying late into the evenings cloistered in his private office-laboratory, developing new and ever more complicated fly stocks to answer still-unresolved mutation questions, and writing book chapters, papers, and speeches at a rate that multiplied after the Prize. The lab then had several research associates, four or five graduate students, and a few laboratory technicians. We students would often try to corner him for questions in the evening when he was in. His light showed only at the door-sill level and we would stoop to see if it was on. This behavior prompted a janitor to ask me why MULLER's students always bowed before his door, even if he was a Nobel Prize winner.

Later MULLER used Thursday afternoons for graduate student appointments to discuss research problems. There would often be pitched battles in which we defended our results and interpretation as best we could against his piercing criticisms. However, when either our experiment or his (if one was a research assistant) failed, he was mercifully kind and supportive. At the time I was preparing my thesis draft, he went over it with me line by line and by the third or fourth draft he was editing his previous suggestions. When I switched from cheap yellow to white bond paper, he decided it was acceptable, probably because he didn't want to waste expensive paper. For his 65th birthday party his students prepared songs and poems about him. A few days later, they came back edited.

When he went on sabbatical to Hawaii in 1953, JIM TELFER and I were assigned to do his neutron experiments. By frequent mail we received his ever-changing recommended fly crosses, which we would then expand in order to collect the necessary thousands of flies. Usually half-way through, he would decide on an alternate procedure and a mad scramble would ensue to build up the stocks before we drove from Bloomington to Oak Ridge for the neutron irradiation. TRACY SONNEBORN likened MULLER to Sturm und Drang, and this atmosphere was most apparent when you were the teaching assistant in his genetics laboratory course. Heaven help you if the matings or the virginal flies were not available on time for the Monday and Friday classes.

Two more memories (J.F.C.): I first met MULLER in the early 1940s when he was at Amherst College. I was then teaching naval trainees at Dartmouth College, not far away, so we had frequent visits. Of course, I was greatly stimulated by MULLER's deep knowledge and creative mind. Once I happened to visit him during yet another low point in his life, just after he had been notified that his temporary appointment could not be made permanent. I recall being greatly incensed that this great man, in the eyes of many our greatest geneticist, didn't have a job. Some thought he was a communist since he had spent much time in Russia. Others of leftist persuasion called him a fascist, for by this time he was denouncing LYSENKO and Stalin. He laughingly said that at least both could not be correct.

MULLER was very active in the Humanist Society and was once its president. In 1963 he was named "Humanist of the Year" and it was my honor to read a citation. In those days, manned space flights had begun and MULLER was very excited. In fact, in his evolution course he spent much time on the origin of the universe, of the solar system, and of life. Knowing this, I said: "If it were possible to send a man to Mars and bring him back safely and quickly, my candidate would be H. J. MULLER. For one thing, his spirit of adventure is such that he would enjoy the trip. But more important, he would have more interesting, exciting, and scientifically important observations to report than anyone I can think of."

I could see him beaming as I read it.

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