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Ryan:

This is a new paper....just published today....it is a very detailed criticism of the LNT model. The policy people might find it useful.

Ed

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Flaws in the LNT single-hit model for cancer risk: An historical assessment

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ABSTRACT

The LNT single-hit model was derived from the Nobel Prize-winning research of Herman J. Muller who showed that x-rays could induce gene mutations in Drosophila and that the dose response for these so-called mutational events was linear. Lewis J. Stadler, another well-known and respected geneticist at the time, strongly disagreed with and challenged Muller's claims. Detailed evaluations by Stadler over a prolonged series of investigations revealed that Muller's experiments had induced gross heritable chromosomal damage instead of specific gene mutations as had been claimed by Muller at his Nobel Lecture. These X-ray-induced alterations became progressively more frequent and were of larger magnitude (more destructive) with increasing doses. Thus, Muller's claim of having induced discrete gene mutations represented a substantial speculative overreach and was, in fact, without proof. The post hoc arguments of Muller to support his gene mutation hypothesis were significantly challenged and weakened by a series of new findings in the areas of cytogenetics, reverse mutation, adaptive and repair processes, and modern molecular methods for estimating induced genetic damage. These findings represented critical and substantial limitations to Muller's hypothesis of X-ray-induced gene mutations. Furthermore, they challenged the scientific foundations used in support of the LNT single-hit model by severing the logical nexus between Muller's data on radiation-induced inheritable alterations and the LNT single-hit model. These findings exposed fundamental scientific flaws that undermined not only the seminal recommendation of the 1956 BEAR I Genetics Panel to adopt the LNT single-hit Model for risk assessment but also any rationale for its continued use in the present day.

1. Introduction

On an August day in 1946 at the University of Rochester, the geneticist Ernst Caspari reported to his supervisor, Curt Stern, that his experiment on gamma-ray-induced mutations in Drosophila had produced results that neither scientist had anticipated. Caspari had fully expected to replicate the 1944 study of Ray-Chaudhuri who had shown that total X-ray/gamma-ray dose was the best predictor of mutational events in mature Drosophila spermatozoa. This study was important because it provided evidence that the dose response for ionizing radiation-induced mutations was cumulative, irreversible, independent of the dose rate, and linear at low doses. However, in contrast to Ray-Chaudhuri's earlier results, Caspari found that the dose rate rather than the total dose determined the dose response and that for a sufficiently low dose rate no response could be detected, revealing an apparent threshold response for gamma-ray-induced mutations. The Caspari studies were part of the Manhattan Project and as such were generally stronger scientifically than those of most independent, single researchers, including the studies of Ray-Chaudhuri. For example, as compared to the Ray-Chaudhuri study, the Caspari study had a more robust design, used larger numbers of flies, exerted superior oversight and guidance, applied better quality control, and assessed a notably lower dose and dose rate, amongst other factors (Calabrese, 2011; Caspari and Stern, 1948).

The Caspari data produced considerable alarm and concern throughout a radiation genetics community fearing that the LNT single-hit model might not now replace the currently accepted threshold dose-response model. The reactions of some leading radiation geneticists to the Caspari findings have been documented and published (Calabrese, 2011, 2015a). One especially succinct and notable response came from Milisav Demerec who asked of Caspari, "What can we do to save the hit model" (Calabrese, 2011). The above cited papers document prestigious members of the radiation geneticist community, including Nobel Prize recipients and an expert Genetics Panel of the U.S. National Academy of Sciences, who saved the "hit" model by obfuscating, deceiving and misrepresenting the scientific record. Sadly, these ideologically based and scandalous manipulations of the scientific record (Calabrese, 2015a, 2015b, 2017a) proved highly successful; they quickly infiltrated and affected the highest levels of regulatory policy in the US Government where they still remain in place today.

The present paper examines a surprisingly under-researched and critical aspect of the cancer risk assessment process by seeking answers to the

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following questions: (1) what was the genesis of the LNT Single-hit Hypothesis, (2) what was its scientific basis and (3) how and when was it validated? To address these questions it is necessary to re-examine the discovery of X-ray-induced heritable mutations by Hermann J. Muller, to assess the scientific validity of Muller's findings and interpretations and, finally, to understand how the scientific community reacted to and accepted Muller's personal account of his results. Acceptance by the scientific community essentially affirmed a biological basis for the Single-hit Model and, at the same time, reassured the public of its "legitimate" use in the assessment of cancer risk for decades to come.

Lewis J. Stadler, a plant geneticist of comparable standing to Muller within the radiation geneticist community, challenged Muller's 1927 claim of having induced gene mutations with X-rays and, as a result, initiated a fierce data-driven debate that only ended much later with the 1954 death of Stadler. Although Muller's views would eventually predominate, the experimental data and arguments offered by Stadler were formidable. The 25-year Muller-Stadler debate generated a unique scientific record that has never been used to re-evaluate the LNT single-hit model and, therefore, validate its application in the process of cancer risk assessment. This is quite astonishing considering that the LNT single-hit model would emerge primarily from the decades-long Muller-Stadler debate. Although Muller's views eventually prevailed, the questions raised herein are why they prevailed, should they have prevailed and, if not, then what does this mean for us today? This assessment concludes that the arguments of Stadler were based on stronger data and were more scientifically persuasive than were Muller's. Yet, in spite of Stadler's superior arguments, his views would show little penetration and have no impact on risk assessment policy.

The story of the LNT single-hit model is complicated and has numerous twists and turns. In the end, however, this new assessment enables a better clarification and understanding of the LNT single-hit model. It challenges the nearly universally accepted view that Muller, in his key groundbreaking experiments of 1927 (Muller, 1927a), had induced the "artificial transmutation of the gene" (i.e., induced "true" mutations) with high doses of X-rays. For it is now known that the vast majority, perhaps all, of the heritable phenotypic changes that Muller had observed were due to substantial non-gene alterations consisting mostly of small, large and massive chromosomal deletions. Furthermore, the LNT single-hit model made critical assumptions about mutations and their dose and dose-rate dependencies that were incorrect. The LNT single-hit cancer risk-assessment model, as it turns out, is the product of a sequence of historical myths about Muller's Nobel Prize findings. Amazingly, these myths mesmerized the scientific community even in the face of objective contemporary scientific challenges that have since been validated with modern analytic tools for mutation assessment. The historical findings presented herein demonstrate that the LNT single-hit model is scientifically flawed and also that the cancer risk assessment policy, which is based on the LNT single-hit model, is equally flawed.

2. Muller and the discovery of X-ray induced mutation

On July 22, 1927, Muller published a paper in the journal Science making the then striking claim that X-rays can induce heritable mutations in fruit fly gonads. However, because such a groundbreaking paper was published without data, some scientists, including his Ph.D. mentor T. H. Morgan (Carlson, 1981), were rightly skeptical and questioned whether Muller had the data he claimed.[1] Apparently, the extraordinary editorial decision at Science to publish such a significant paper without requiring data has yet to be explained some 90 years later. Two months after his Science publication, at the 5th International Genetics Congress in Berlin, Muller finally showed the world convincing evidence that X-rays induced heritable mutations in fruit fly gonads.

Muller (1927a, 1928a) used the method of sex-linked recessive lethality in the genomes of male and female fruit flies to demonstrate X-ray-induced mutations. To distinguish his findings from those of researchers who induced heritable "chromosomal" changes, including various types of aberrations (e.g., deletions, insertions), non-disjunction and other gross chromosomal alterations, Muller emphasized that he had artificially altered the "gene", a much smaller unit of inheritance than the "chromosome". In his first experiment Muller produced an apparent threshold dose response using four doses and a standard Drosophila strain. Switching to his newly developed C1B strain and reducing the number of doses to two (i.e., doses #2 and #4), Muller showed that mortality was enhanced by some 150 fold at the highest dose when results from the two experiments were combined. Although the findings were limited, the data suggested a non-linear (square root), dose-response function (Muller, 1927a). A broad spectrum of visual phenotypic changes was also reported, indicating that the X-ray treatment had affected many genes.

While it is generally believed that Muller had won the race to be first in inducing gene mutations by a scant three months,[2] narrowly beating several groups of plant geneticists from the US, including Lewis Stadler[3] from the University of Missouri and Thomas Goodspeed from the University of California at Berkeley, he may actually have come in second. Charles Stuart Gager (Brooklyn Botanical Gardens) and Albert Blakeslee (Carnegie Institute for Research) were the first researchers to report in the Proceedings of the US National Academy of Sciences in January of 1927 that ionizing radiation induced gene mutations in Jimson Weed (Datura).[4,5,6] Their paper revealed that radiation induced

[1] Carlson (1981) (pages 148–149) stated that T.H. Morgan believed that Muller had finally "hung himself", having recalled an earlier incident in which Muller overstated that the Drosophila spontaneous mutation rate for the X-chromosome was 1%. Morgan held there was little chance to escape the apparent exaggeration of the so-called 15,000% increase in X-ray induced mutation rate. The statement of Morgan reveals that he was reluctant to trust the assertions of Muller while also having reservations about his character. The lack of professional and perhaps personal regard of Muller may be seen when Morgan sponsored Sturtevant for membership in the NAS, rather than Muller, some five years after Muller's discovery of X-ray induced mutation (Carlson, 1981, page 174).

[2] Stadler presented his mutational evidence in Nashville, Tennessee, at the AAAS meeting in December of 1927. Stadler had treated barley seeds with X-rays in 1926, collecting the subsequent seeds later that season. These seeds were planted in the next growing season (1927). The findings revealed evidence of mutation. While 1300 tiller controls showed no trans-generational phenotypic changes, the X-ray treatment yielded 14 mutations in 1200 tillers (Carlson, 1981, page 151).

[3] Rhoades (1984) has emphasized that Muller and Stadler had a different goal/approach to their respective X-ray induced mutation research. In the case of Stadler, he employed X-rays to induce mutations to study gene structure and the nature of gene mutation alterations. Thus, Stadler focused on mutations at various loci that mediated specific phenotypic functions. In contrast, Muller focused on the spectrum of induced mutational changes.

[4] While Gager and Blakeslee would assert the primacy of their gene mutation discovery over the next 15 years (Blakeslee, 1940, 1942; Gager, 1931, 1936), they acknowledged that Muller had developed a method for generating large numbers of mutations. As primacy was a critical issue for Muller, he would routinely attempt to discredit the claims of others, including Gager and Blakeslee. In retrospect, the assertion of Gager and Blakeslee has withstood the criticism of Muller (Campos, 2015). However, the assertions and claims of Gager and Blakeslee would generally be ignored while Muller's findings would capture the excitement of the scientific community (Campos, 2015).

[5] Stadler (1936) would recognize the primacy of Gager and Blakeslee and their mutational findings. He specifically stated that: "Before the publication of Muller's results, several investigations of the genetic effects of penetrating inductions in plants were in progress and some positive results were obtained. The work of Gager and Blakeslee (1927) is especially noteworthy in this connection". Of the 113 progeny plants from parents treated with radium, 20 were chromosomal variants. Amongst these chromosomal variants two were heterozygous for new recessive genes. Even though the number of gene mutations was small, Stadler (1936) considered the findings of considerable importance. The publication of the Gager and Blakeslee (January 14, 1927) preceded the Muller note in Science, (i.e., July 22, 1927) which lacked any data, by more than six months. Despite his recognition and appreciation of the findings of Gager and Blakeslee (1927), Stadler (1954) would argue that they too fell short of "conclusive" proof due to inadequate statistical evidence and failure to show that the genetic changes reported were the result of the treatment and not to some type of genetic irregularity of the treated strains.

[6] Muller (1928a) would present his findings at the Fifth International Genetics Congress in Berlin (September 1927). At the same Congress Blakeslee (1928) would also

a few apparent "real" gene mutations. Muller's principal achievement, on the other hand, was finding a methodology to enhance the production of what were believed to be unequivocal gene mutations. Conflating the methodology with the discovery helped create the notion and then the myth (Campos, 2015) that Muller produced the first artificial transmutation of a gene as well as the technique to vastly increase their production. More extensive assessments of the dose response would follow and expand Muller's basic observations in support of a linear dose response (Oliver, 1930; Hanson and Heys, 1929).

3. When is a mutation a "True" mutation?

Despite the consistently reproducible findings that X-rays could induce heritable mutations, questions began to arise as to whether Muller had actually induced "true" mutations.[7,8] Shades of doubt surfaced at the University of Missouri with Lewis J. Stadler's detailed cytogenetic evaluations of the effects of X-rays on maize. In a 1931 publication, Stadler began to draw a distinction between those changes that occur in the individual gene and those that relate to gross chromosomal alterations (e.g., deletions, reduplication or rearrangement of unchanged genes). Although this distinction, as Stadler saw it, was obviously important due to the role of gene mutation in evolution, gross alterations resulting from chromosomal irregularities were just as heritable[9] and therefore of no less importance.

Stadler had a preeminent scientific reputation; he was a highly regarded plant geneticist, a member of the U.S. NAS and the President of the Genetics Society of America (GSA) (1938). As such, his curiosity to differentiate between gene and chromosomal alterations was not only significant but also central to his research program on understanding the nature of the gene. He collaborated with the future Nobel Prize (1983) recipient Barbara McClintock and then recruited her to assess the effects of X-rays on maize chromosomes at the University of Missouri. McClintock's expertise proved immensely valuable, as she had just developed the capacity to markedly improve chromosomal staining of the pachytene stage of prophase.[10] So, McClintock began to assess Stadler's maize chromosomes in the summer of 1931. In her own words to the Nobel audience (McClintock, 1983) she described her research in Stadler's laboratory:

> "…It was to observe the effects of X-rays on chromosomes of maize that brought me to the University of Missouri in 1931.... Irradiated male gametes in Stadler's experiments carried wild-type alleles of known recessive mutants…An X-ray induced mutation altering the expression of the wild-type allele of one of these recessives should be identifiable in an individual plant derived from such a cross.…-following my arrival at Columbia in June 1931, plants were selected where chromosomes were to be examined." (Meiotic stages were examined to look for certain kinds of events that might be responsible for the expression of recessives)…None of the recessive phenotypes in the examined plants arose from "gene mutation". Each reflected loss of a segment of a chromosome that carried the wild-type allele, and x-rays were responsible for inducing these deficiencies. They were also responsible for producing other types of chromosomal rearrangement, some of them unexpectedly complex.…"

The collaboration between Stadler and McClintock affected Stadler's understanding of the problem almost immediately. His presentation in 1932 at the Sixth International Congress on Genetics at Cornell University challenged Muller's assertion of having induced gene mutation.[11] Furthermore, in his 1933 annual report (Stadler, 1933) to the National Research Council, Stadler stated that "investigation of the physical nature of the induced 'gene mutations' indicates that these are chiefly or wholly the result, not of changes within the gene, but of extragenic alterations of various kinds". Table 1 provides quotations from several of Stadler's publications (from 1932 to his final publication in 1954) that challenged Muller's assertions that he had observed "true" gene mutations. With the arrival of new analytic tools since the mid-1970's, such as the Southern blot (Southern, 1975), PCR (Mullis and Faloona, 1987) and precise DNA sequencing technologies, others in the research community extended this debate with much greater specificity and clarity.

Stadler's views on the nature of X-ray-induced genetic damage seemed to crystallize following new developments in cytological techniques that included Painter's salivary gland method (Painter, 1933, 1934a, 1934b, 1934c) in Drosophila and McClintock's earlier pachytene technique in maize (McClintock, 1929a, 1931) and their applications in the analysis of X-ray induced mutations.[12] As a result of the applications

(footnote continued)
summarize the mutation findings of Gager and himself as published earlier (Gager and Blakeslee, 1927). In the case of Muller, he seized the moment and captured the fascination of his audience; whereas Blakeslee would submerge/underplay his novel mutational findings in an article obscurely entitled "The Genetics of Datura". The failure of Gager and Blakeslee to promote their key findings was in striking contrast to the demonstrable Muller. Elof Carlson (1981), Muller's biographer (page 151), incorrectly stated that Blakeslee confirmed the X-ray-induced mutation results of Muller within a year after Muller's report. As documented here, Gager and Blakeslee (1927) published their data a year ahead of Muller's (1928a, 1928b) data in the conference proceedings and six months prior to Muller's data-free Science paper.

[7] A key aspect of the Muller story was his ability to redefine the contemporary view of what constituted a "real" mutation (Campos, 2015). Prior to Muller's engagement on this issue the generally accepted view was that inherited phenotypic changes were considered mutations. This could occur via alterations with the chromosome and/or gene. Muller would be unrelenting in his quest to drop chromosomal inheritance from the definition. So focused was Muller on winning this conceptual and terminological argument that he utilized the title "artificial transmutation of the gene" to describe the mutation he believed he had induced by X-ray treatment (Muller, 1927a).

[8] Prior to the discovery of McClintock, cytological evaluation of radiation-induced chromosome aberrations/changes were limited to the condensed metaphase chromosomes of somatic cells. In such a setting it is only possible to identify gross effects, including changes in chromosome number or large translocations or losses of chromosome sections. McClintock's contribution involved the capacity to study thin-stranded chromosomes of early prophase of meiosis, permitting a far more precise documentation of radiation-induced changes than had occurred previously. In maize, the chromosomes are approximately 10-fold longer during prophase than they are at metaphase and the homologous chromosomes are closely aligned. Thus, the loss of even a very small part of the chromosome is easily observed. Likewise, the nature of translocations/damage is therefore markedly more refined and could then be used with greater precision to assess chromosomal alterations at far higher resolution and at far lower doses of radiation.

[9] For Stadler (1954) the error of Muller in the debate was that he combined his observed effect (i.e., heritable phenotypic changes) (Step 1) with an interpretation that the observed effects were due to a specific mechanism (Step 2). Stadler (1954) emphasized that these two steps be kept distinct since Step 1 is a specific contribution to scientific knowledge, while the second is based on an inference/interpretation. By combining the two, the scientific community may confuse what it knows with what it thinks it knows. Stadler (1954) asserted that the belief that the frequency of gene mutation may be profoundly increased by X-ray treatment was "an illusion of this kind". The problem emerged from an insistence that X-rays induced gene mutations, since the mutants that are formed satisfy all the criteria of gene mutations (e.g., heritable, reversible) and that these mutants represent qualitative alterations in specific genes, since that is the definition of a gene mutation. In effect, Stadler argued that the error resides in using the same term for two concepts. This terminological combination then becomes a substitute for a bonafide scientific mechanism of proof - the trap to which the mutation field was vulnerable.

[10] The technique of McClintock (1931) for the assessment of maize chromosomes in prophase of meiosis made possible the cytological identification of minor structural chromosomal changes with a precision far exceeding that possible in studies using condensed chromosomes. The breakthrough methodological development of McClintock (1929a, 1929b, 1931) forced Stadler to realize that viable deficiencies were able to account for some of the apparent/presumed gene mutations induced by X-ray treatment. Thus, for Stadler (1931) it was "now clear that many of the variations identified by their genetic behaviors as gene mutations are due to mechanical alteration analogous to the grosser chromosomal aberrations.".

[11] Note that Stadler's presentation at the Sixth International Congress on Genetics was one of only three Plenary addresses on mutagenesis (Haynes, 1998). With Muller in the audience, Stadler had clearly offered a major public challenge to Muller's interpretation.

[12] Painter developed an application of the acetocarmine smear method that had been employed by cytogeneticists working on maize chromosomes (Glass, 1990). According to Carlson (1981), Muller first learned of Painter's (1933) advance while in Leningrad. He immediately understood its significance for the assessment of X-ray-induced small rearrangements and chromosome breaks.

Table 1
Stadler's Challenge to Muller.

Stadler, L.J., 1932. Proc 6th Int. Cong Genet. 1, pp. 274–294 (Stadler, 1932)
"To state that an induced variation is a gene mutation is not to explain it but merely to label it."
Page 274–275
"We do not demonstrate that a chemical change has occurred; we simply infer, since no mechanical explanation can be found, that the variation must be due to this invisible mechanism."
Page 275
"We may define mutation as a transmissible change in the gene. But we identify mutation by experimental tests, and these tests are not such as to establish conclusively, in specific instances, that a change within the gene has occurred."
Page 275
"In effect, any Mendelizing variation which cannot be shown to be due to a change involving more than one gene is a mutation."
Page 275
"Thus the working definition of mutation necessarily differs somewhat from the ideal definition. It is this working definition which must be considered in generalizing from the experimental evidence. The mutations experimentally known may include not only variations due to alterations with the gene but also variations due to losses of genes or groups of genes, to additions of genes or groups of genes, and possibly also to changes in the spatial relationships of genes to one another."
Page 275–276
" ...the parallel between induced mutations and induced chromosome derangements is striking."
"The frequency of induced mutation is directly proportional to dosage (Stadler, 1928, 1930). So are the frequency of deficiencies induced by treatment of pollen (Stadler, 1931) and the frequency of endosperm mosaics induced by treatment shortly after fertilization (Goodsell, 1930).
Page 282
"The induced mutation also may involve various types of germinal change, for there are several conceivable ways in which irradiation may so modify a chromosome or to cause the appearance of a Mendelizing variation."
Page 291
" ....most of the induced mutations in plants are due to various extragenic alterations, chiefly non-lethal deficiencies. This is true in spite of the fact that the variation record as mutations in the experiments with plants are exclusively the "visible"...: "
Page 291
"The frequent occurrence in Drosophila of mutations at the points of breaks of the chromosomes suggests that the point mutations in this species as well as in plants may be largely of mechanical origin. This association of induced mutations with chromosome breakage does not necessarily exclude the possibility that the mutations are intra-genie changes, for it is conceivable that some change within the gene may be the cause of the break, and that in some instances the gene may continue to function as a gene after the change has occurred. But the possibility that some mutant characters may be due merely to the changed spatial relations of gene to another ("position effect") can not be disregarded."
Page 292
"....the occurrence of reversion is not proof that the original mutation could not have been due even to a deficiency."
Page 292
Source: Stadler, L.J., 1936. Duggar Editor, pp. 1263–1280 (Stadler, 1936)
"Mutation implies a hypothetical change within the individual gene, or at any rate a change affecting no than more than a single gene. But since the individual gene is invisible, the identification of a germinal variation as a mutation is a matter of inference we assume that it is due to a change in the gene."
Page1263
"Since chromosomal alterations (particularly small deficiencies) are known to simulate gene mutation in some instances, and since irradiation regularly induces chromosomal alterations (including deficiencies), there is obvious ground for the suspicion that the apparent gene mutations induced by irradiation are in fact due to extragenic alterations."
Page 1275
Source: Stadler, L.J., 1954. Science 120(3125), pp. 811–819 (Stadler, 1954)
"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)".
Page 813
"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)."
Page 813
"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 would be the result of a translocation affecting the expression of the unchanged gene."
Page 818

of such new cytological techniques in experimental studies, it was found that most of the X-ray-induced heritable changes produced at high doses were due to gross chromosomal aberrations or extragenic changes. In fact, the cytological advance by Painter (1934a, 1934b, 1934c) would profoundly affect the debate between "gene" and "chromosomal" mutations. So significant was this methodological development that Science published a brief summary note by Painter (1933) prior to his three papers in Genetics in 1934 (Painter, 1934a, 1934b, 1934c). Just as the earlier advances by McClintock (1929a, 1929b) had affected Stadler's view, so too did Painter's application influence the view of the Drosophila research community.

According to Carlson (1981), by the end of 1931 Stadler's criticisms of Muller's gene mutation claim seemed to be causing Muller some distress. Upon returning in December of 1931 from a conference of the American Society of Naturalists in New Orleans, Muller was showing stress on multiple fronts. His claims of radiation-induced mutations were being more broadly challenged and his once productive relationship with Patterson was now becoming one of confrontations and lack of trust. Likewise, his collaboration with the cytogeneticist Painter fell apart in 1929, again amidst distrust and professional jealousy. During this same period, Morgan would bypass Muller for an NAS nomination, favoring his former fellow graduate student, Sturtevant.

So penetrating and persuasive were the criticisms of Stadler that Muller's own convictions in the "artificial transmutation of the gene" were beginning to erode. Campos (2015) (see page 327, footnote 168) confirmed "it was Muller's discussions with Stadler and others that would ultimately cause Muller to begin to question the viability of a firm distinction between point mutations and exceptionally minor chromosomal rearrangements, as well as the nature of the position effects." Stadler's criticisms would follow Muller on his journeys from Texas to Germany to the Soviet Union and back to the U.S. Eventually Muller would recognize that ionizing radiation produced a vast abundance of major chromosomal aberrations at high doses. More importantly, his colleague Mark Belgovsky discovered that even small chromosomal rearrangements were readily induced by ionizing radiation and displayed a linear dose-response relationship (Belgovsky and Muller, 1938). These findings were of significance since they indicated that most of the induced gene (i.e., real) mutations at these lower doses were small-modest deletions or inversions.

Despite all the evidence that was being amassed against his 1927 "gene" mutation assertion in Science, Muller would cling to this idea because he thought that the mechanism of evolution could not involve heritable chromosomal mutations. Although the logic is sound that minor, intragenic mutations are the tiny engines of evolution, Muller was wrong in applying this logic to interpret the effects produced by high doses of X-rays.

In 1932, Muller left his position at the University of Texas (where Painter was a professor) to research with Timofeeff-Ressovsky in Berlin. At the end of about two years (and about the same time that Painter completed the development of his cytogenetic staining technique in Texas), Muller moved to the Soviet Union to direct a genetics research program. Muller continued to promote his arguments on gene mutation (Muller, 1935, 1939a, 1939b) in both Berlin and Leningrad/St. Petersburg and Moscow. Meanwhile, the cytogenetics tools developed by

Painter, his former colleague, were being established and used to acquire evidence that challenged the acceptance of X-ray-induced gene mutations at high doses, representing a formidable obstacle to Muller. Painter's publications would also undermine the research of Timofeeff-Ressovsky et al. (1935) who were in the final stages of integrating two concepts: the LNT single-hit model and the mutation of "genes" with high doses of X-rays. Essentially, the new cytogenetics techniques of McClintock and Painter were yielding more refined data that was eroding support for the LNT single-hit model and, at the same time, shifting the discussion toward Ray-Chaudhuri's ideas of total-dose and linearity to dose rate and nonlinear threshold.

Meanwhile, Stadler undertook an extensive research program that evaluated X-ray-induced alterations at specific gene loci in maize, including possible changes within the gene itself. These experiments included visible assessments of chromosomal aberrations, transmission of chromosomal mediated changes through male/female germ cells, viability of the homozygote, and mutation reversion back to the non-mutated gene. Throughout his entire 25-year research effort, it is highly significant that Stadler would never find experimental evidence to support X-ray-induced gene (i.e., "real") mutations.

As research progressed, several developments emerged as significant. One involved the discovery of a position effect in Drosophila. This occurred when an X-ray-induced chromosomal aberration affected the translocation of a gene from one locus to another. With movement to the new locus, the transferred gene was found to affect activity in neighboring gene(s), even though the gene(s) itself was unaltered in its composition. If the transferred gene were physically returned to its original locus the organism would then revert back to its normal wild type. Interestingly, this genetic behavior was considered to have satisfied the criteria for reversibility, despite the fact that a "gene" had not been truly mutated, i.e., no change had occurred in its sequential nucleotide composition.

A second research development emerged as significant. It involved drawing the same conclusion (that X-rays induce gene mutations) from numerous studies based on an unproven and untested assumption (that phenotypic change equals compositional gene change). That is, it was assumed that an X-ray-induced phenotypic change could only result from the compositional alteration of a gene and that no verification of that assumption was apparently necessary. Missing was a detailed proof by the investigator that an X-ray-induced phenotypic change produced a corresponding alteration in the composition of a gene related to that specific phenotype. Also missing were more critical and detailed reviews of the publications to prevent uncontested and unproven assumptions from leading to false conclusions, wide acceptance and eventually erroneous dogma masquerading as theory. According to Nuffer (1957), a large number of such insufficiently assessed publications led to the belief that X-ray treatments were inducing gene mutations. However, when specific cases were examined more closely, some type of chromosomal aberration usually explained that which was assumed to be a gene mutation.

4. Muller's arguments to support the gene mutation hypothesis

To preserve the uniqueness and significance of his favored concept—the artificial transmutation of the gene, Muller published an 82-page paper in 1930 with his University of Texas colleague J.T. Patterson (Patterson and Muller, 1930). The assessment centered on whether the X-ray-induced trans-generational phenotypic changes in Drosophila were due either to losses (deletions), rearrangements of portions of chromosomes or to the so-called "progressive" point-like genetic changes that Muller assumed to drive evolution. This article reflected patterns in Muller's professional life; that is, he marshaled as much evidence as possible, presented it in excruciating detail, and never compromised on an essential point (i.e., classic example of a "Mullerism"). Also reflected in the paper was Muller's own perception of the fundamental weakness in his interpretation of a gene mutation, which occurred a year prior to the emergence of Stadler's (1931) questioning of this basic tenet.[13]

Patterson (Muller's Department Chair at UTexas) and Muller (1930) argued that the induced gene mutations are generally the direct result of relatively minor local electronic "hits" rather than more destructive processes (i.e., the gene versus chromosome mutation debate). The reasons for their stated beliefs included: (1) the general randomness and specificity of induced phenotypic changes (i.e., Muller's gene mutations); (2) identical phenotypes were independently affected; (3) phenotypic changes were dose dependent; (4) numerous toxic chemicals were not effective in producing such changes and (5) "most important of all, probably, is the fact that a direct and simple proportionality has been shown to exist between the frequency of the induced mutations and the amount (energy) of the radiation absorbed." They cited Hanson and Heys (1929) and Oliver (1930) to support this conclusion and then stated that "there is no indication in the results of any lower critical intensity, or threshold value, beneath which there is no (or a relatively lesser) effect." They concluded with "All these facts and considerations converge to indicate strongly that the mutations are rather direct effects of individual quantum-absorption electron hits…". Several years later, Oliver (1934), who received his Ph.D. in 1931 (Oliver, 1931a) while working under the direction of Muller, further expanded this list of arguments in favor of gene mutations. He indicated that X-ray-induced mutations often seem to appear similar to spontaneous mutations, an issue that would be re-examined at each stage of technological advance. Within this framework Oliver (1934) noted that the proportion of lethals to visibles or of dominants to recessives is similar to those reported for the spontaneous mutation rates (Morgan et al., 1932; Muller, 1930a, 1930b). Further supporting this perspective were observations that the frequently mutating genes in the spontaneous state are the same genes that frequently mutate when treated with X-rays.

In addition to this list of arguments in support of the gene mutation hypothesis, Muller would rely most heavily on two other linked arguments: (1) the occurrence of reverse mutations and (2) the multigenerational inheritability of mutations. Muller argued that if a mutation could be reversed then the gene had not been destroyed or deleted. Such reasoning led Muller and his supporters to conclude that "real" gene mutations are induced by the direct activity of X-rays.

Early reaction against such reasoning surprisingly came from one of Muller's own students. In the conclusion to his 1931 dissertation, Oliver (1931a) stated, "it is not possible to produce gene mutations without producing gene rearrangements". Later, Oliver also observed that X-ray-induced "inversions and translocations were produced in direct proportion to the intensity of treatment" (Oliver, 1934), a similar observation that had been previously used to support gene mutations. Oliver's proportionality observation for the induction of gross chromosomal damage proved to be consistent with later observations of other researchers (Timofeeff-Ressvosky, 1939; Demerec, 1937). Oliver (1934) also reported that translocations are induced with X-ray treatment of Drosophila sperm (Muller, 1928a, 1927a, 1927b; Weinstein,

[13] Within but three months of his momentous presentation in Berlin in September 1927 demonstrating X-ray induced heritable mutations, Muller (1927c) read a paper at the AAAS Conference in Nashville, Tennessee. During this presentation he addressed for the first time the possibility that his "gene mutations" were not gene mutations after all, but the results of chromosomal aberrations. He defended the gene mutation hypothesis by introducing the "reverse" mutation explanation, which he suggested provided a mechanism for evolution. Four months later Muller read a paper before the U.S. NAS (April 24, 1928) (Muller, 1928b) in which he raised the question "Are the X-rays merely punching holes in the chromosomes, or, more precisely stated, simply causing losses or inactivation of genes or gene parts? If so, the usefulness of the X-ray, both from a practical and from a theoretical standpoint in biology, would be seriously limited (emphasis added)." Muller therefore clearly understood that his X-ray-induced transgenerational phenotypic changes would only be significant when due to intra-genic/small point mutations. Muller stated that he was challenged to confront this issue by his former graduate student colleague and lifelong friend, Edgar Altenburg. Thus, the seeds of self-doubt on this issue preceded Stadler's own dissatisfactions with Muller's gene mutation interpretation by some three years.

1928) with a frequency equal to that of "gene" mutations (Muller, 1928a; Muller and Altenburg, 1928; Muller and Altenburg, 1930; Oliver, 1931b; Whiting and Bostian, 1931). In his own research, Oliver (1934) recorded 21 lethals, 16 crossing-over conditions with most being inversions, and 17 translocations occurring in 105 subjects from the high dose parents (t_{16}, 4560 r), and 18 lethals, 13 crossing overs and 10 translocations, all with the X-chromosome in 400 offspring of parents treated with a lower dose (t_4, 1140 r). On page 391, Oliver (1934) stated, "it is probable that all lethal mutations are not point mutations, but that some are connected to, and due to chromosomal aberrations (Muller, 1927a; Weinstein, 1928) of definite deficiencies (Patterson, 1932)." These lethals, which are connected to chromosomal aberrations, increase rapidly with high doses (Oliver, 1930, 1932). On page 392, Oliver (1934) stated, "that X-rays increase the frequency of inversions as well as lethal mutations in Drosophila", as has been reported by Serebrovsky (1929), Muller (1927a, 1927b, 1928b), Oliver (1930) and others. The direct proportionality of the frequency of inductions of sex-linked inversions and translocations for Drosophila was determined by Oliver (1931b, 1932) and deficiencies in maize by Goodsell (1930) and Stadler (1931)" (page 392). It is curious that Oliver (1934)—Muller's former student—was the one who adopted such an active role in criticizing X-ray-induced gene mutations. It should be noted that Stadler (1932) also reported similar criticisms at the Sixth International Genetics Congress, suggesting that an alternative to Muller's view of discrete X-ray-induced "gene" mutations was emerging and growing stronger.

Oliver's dissertation (1931a) revealed that Muller's 150-fold increase in the frequency of lethal gene mutations at the highest dose was a significant overestimation. Oliver calculated that other damage-related endpoints, such as crossing over and translocations, could easily have accounted for a large proportion of the sex-linked lethality that had been attributed to gene mutation. Furthermore, Muller's estimation of "real mutations" would have decreased even more if Oliver (1931a) had included damage due to deletions and if Muller's control mutation rate had not been so low (< 1/1000). Finally, because the control background incidence of sex-linked recessive mortality ranged broadly over an order of magnitude (Muller, 1927a), it is possible that the variability of controls could have skewed Muller's estimation significantly upward.

Although sex-linked mortality probably increased, the estimated "massive" increase was probably not due to "real" gene mutations, as the scientific community was led to believe. This perspective is important because much of the excitement associated with Muller's findings was derived from the mistaken belief that Muller had produced large numbers of gene mutations.

5. The reverse mutation: a critical issue in the gene mutation debate

The debate between Stadler and Muller would continue with Stadler seeking the consultation of Drosophila experts to test the reverse mutation hypothesis as had been espoused by Patterson and Muller (1930). As a result of Stadler's inspiration, George Lefevre Jr. decided to dedicate his dissertation research to the task of testing Muller's "real" mutation theory. That is, he wanted to know whether Muller's research had produced "true" heritable mutations at the gene level or mostly a spectrum of modest to extremely large genetic lesions.

In reviewing the literature, Lefevre noted that other Drosophila researchers had also been intrigued by this question. Based on a series of experiments Timofeeff-Ressovsky (1939) reported 24 distinct reverse mutations at seven X-chromosomal loci (24 reverse mutations/343,183 genes) as well as 16 reverse mutations at six II-chromosomal loci. Earlier, Johnston and Winchester (1934) had induced 24 reverse mutations at eight X-chromosome loci (24 reverse mutations/713,001 genes). The combined rate constant for reverse mutations in these two papers is 1.3×10^{-8} at a dose of 4000-5000r. Amongst the reverse mutations noted by Timofeeff-Ressovsky (1939) and Johnston and Winchester (1934) are those which were affirmed by breeding confirmation tests, along with those mutations, based on phenotype evidence, that were thought to be reverse mutations but which could not be confirmed due to sterility induced by high X-ray doses. This mutation rate constant of ~ 1 mutation per 10^8 genes at 4000-5000r indicates that the incidence of such X-ray-induced phenotypic changes is extremely low. This X-ray inducing dose also frequently exceeded the maximum tolerated dose, with 60–70% sterility. If one assumes that a linear dose response occurs for each type of X-ray at a reverse mutation rate of 1×10^{-8} then a predicted genotoxic effect should decrease by about another order of magnitude at the lower dose of 250-300r. This would be similar to the lowest dose used by Oliver (1931a), but still several hundred-thousand-fold greater than the background radiation exposure.

Around the same time, several other reports also provided evidence of X-ray-induced reverse mutations. For example, Dubinin and Goldat (1936) reported seven reversals for yellow, schaete and scute characteristics, while Griffen and Stone (1939) and Glembozky (1936) also reported the occurrence of similar reverse mutations. Although results with Drosophila from the above studies were consistent with the Muller perspective, several other studies failed to induce reverse mutations via X-ray treatment. Kaufmann (1942) reported extensive negative data after radiating 25,000 forked and 12,000 white flies with 4000r. Raffel and Muller (1940) irradiated scute-4 scute-8 recombination chromosomes with 3000r and reported finding no non-scute among 50,000 progeny. Demerec (1938) also reported similar negative findings for reverse mutations of forked and white using approximately 100,000 offspring of recessive parents.[14] In addition to negative findings with respect to induced reverse mutation with X-rays, several effects were found to closely mimic such mutations, requiring enhanced precautions by researchers. For example, Lewis (1945) reported that crossing over between "pseudoalleles" might result in an apparent reverse mutational effect.

According to Lefevre (1949), reverse mutation studies need to preclude the possibility that crossing over could induce reverse mutations. Genetic suppressors can also affect the occurrence of reverse mutations and need to be evaluated as well. A third type of confounder could result from the "contamination" of several markers in the irradiated fly stock that might produce spurious reverse mutations. The presence and accidental co-mingling of other fly stocks possessing the same markers except one was possibly a source of this "contamination" and could lead to making incorrect conclusions about reverse mutations.

Given the above historical foundations, Lefevre offered the following perspective:

> "Because of the several mechanisms that can mimic reverse mutations, the early reports of successful induction of reverse mutation in Drosophila should not be accepted unqualifiedly as evidence of the similarity of induced spontaneous mutation."

For Lefevre, the key question had already been posed and tested by Patterson and Muller (1930). They reasoned that if radiation could induce a recessive mutation and also reverse it back to the normal dominant, then it must follow that the induced mutation could not result from a gross loss of genetic material. Patterson and Muller proceeded to test this argument by performing three successive irradiations and inducing the following three genetic changes: the first irradiation mutated a wild type allele (+) to its "forked" (f) mutant form; the second irradiation reverted the "forked" mutant back to the original wild type; and the third irradiation again mutated the wild type back to its "forked" mutation for a second time. The trans-generational

[14] The failure of Demerec to induce a reverse mutation in such a large number of Drosophila would soon lead him to switch to bacteria for his experimental model (Demerec, 1955).

reversibility of such a specific phenotype (+→f→+→f) convinced Muller that these radiation-induced changes were qualitatively the same as those occurring spontaneously in nature and that they indeed represented "true" gene mutations and were not simply the result of gross chromosomal damage, as Stadler had been claiming.

Contrary to the results of Patterson and Muller, however, was the later dissertation research of Lefevre (1949) who found no evidence of reverse gene mutations when using 5000 r to examine 166,000 recessive X-chromosome loci. Follow up studies on somatic tissue also revealed no reverse mutations in experiments involving normalized 600,000 white loci exposed to 5000r. Together these findings led Lefevre (1950) to conclude that a "serious question is thus thrown on the reliability of the early reports of X-ray induced reverse mutation in Drosophila."

That a reverse mutation is proof of a "true" mutation was a debate that would last for nearly two decades in the published record of studies conducted with multiple biological models and inducing agents (De Serres, 1958; Lefevre and Green, 1959; Muller and Oster, 1957; Emmerling, 1955; Nuffer, 1957; Giles et al., 1955; Mottinger, 1970). Technological advancements would eventually supersede the debate and finally provide the insight and proof that Stadler (1932, 1954) was seeking (see the section entitled "The Modern Era"). The issue of reverse mutation would also play a central role in the research of Barbara McClintock who would propose an alternative to Muller's interpretation of X-ray-induced gene mutations.

6. Rejecting Muller: Mcclintock's alternative gene mutation mechanism

The research of Barbara McClintock significantly challenged Muller's (1927a) interpretation of X-ray-induced gene mutation in multiple ways. First, she developed a cytogenetic staining method that revealed detailed structures of maize chromosomes (McClintock, 1929b). In 1931 this new technology enabled Stadler and McClintock to evaluate research used to hypothesize the existence of X-ray-induced gene mutations in maize. Observations with the new cytogenetic techniques visually revealed that the phenotypic changes produced by the so-called gene mutations were actually due to genetic damage at the level of the chromosome (and not the gene) and consisted largely of deletions, translocations and other gross aberrations.

As a result, Stadler re-evaluated his previous findings in barley (Stadler, 1928) and realized he had been wrong in assuming that his observed heritable changes were due to gene mutations, i.e., Muller's so-called "point mutations". In fact, Stadler also soon came to reject Muller (1927a) evidence-free interpretation of his own Drosophila work that claimed a gene mutation mechanism was responsible for X-ray-induced heritable changes.

Later, McClintock (1938) would provide experimental evidence to support an indirect mechanism that could explain the trans-generational phenotypic changes induced by X-rays in Stadler's studies on maize. This mechanism became known as the break-fusion-bridge (BFB) process and it could induce massive chromosomal aberrations. It involved initial X-ray-induced chromosomal breakage, followed by the joining of the broken ends of two chromosomes, each with its own centromere. These joined/fused chromosomes would line up at metaphase and then break as their centromeres pulled in opposite directions, generating genetic damage.

While following up on the BFB discovery, McClintock detected another way to produce gene mutations. The newly proposed mechanism involved regulatory control over gene expression but did not involve altering the chemical composition of the gene (McClintock, 1953). She showed that specific genes could change location/position (i.e., transposition) within the genome and, in so doing, alter the expression of new neighboring genes (e.g., from dominant to recessive and vice versa). These observations, which would eventually result in McClintock receiving the Nobel Prize in 1983, provided a mechanism by which reverse phenotypic changes due to a "gene" (similar to those that Muller initially reported in his, 1930 paper with Patterson) could be obtained without altering the chemical nature of that "gene". These findings were significant because they presented a serious limitation to Muller's radiation-induced gene mutation model.

In October of 1948, Muller corresponded with McClintock and admitted to her that he thought her mutable-gene discovery involving transpositional elements (TEs) was a "magnificent achievement" (Comfort, 2001). He also wrote her stating "what a relief it is for us [I presume he actually meant himself] to know that mutable genes are a different class from the ordinary gene mutations after all." (Muller, 1948). Taken together, these statements affirmed that Muller recognized the significance and validity of McClintock's discovery but, at the same time, wanted his own hypothesis on radiation-induced gene mutations to be seen as separate and different from her discovery on mutable genes. There seems to be little question that Muller was concerned with how McClintock's discovery might be used to interpret his hypothesis of radiation-induced gene mutations. According to Comfort (2001), Muller had attended a seminar in October of 1948 on McClintock's work that was given by Salvadore Luria (1969 Nobel Prize recipient), a scientist who was greatly impressed with her work and thought it to be extremely important.[15] In his biography of McClintock, Comfort (2001) suggested that Muller had heard only what he wanted to hear at Luria's seminar because he was trying to "save" his gene mutation theory. However, McClintock, who saw her theory as an alternative to Muller's gene mutation model, would have none of it.[16] Muller's model assumed that a gene is an autonomous genetic unit in a string of genes and that a mutation was a discrete break occurring in a single gene on the string of genes. According to Comfort (1997, page 190), McClintock's "mutable genes, which mutate and then 'unmutate' again, are difficult to explain by this [i.e., Muller's] model." Although McClintock's mutable genes model could perhaps be seen as a complement to Muller's model, McClintock saw her model as superior in accuracy, scope, flexibility and integration and, therefore, as a compelling alternative to Muller's model. In fact, Comfort (1997), page 191) noted that McClintock was approaching the conclusion that "all mutations, including X-ray or chemically induced mutations, were the result of reversible alterations via the process of an integrative genome in which genes acted in suites, controlled by regulatory elements." In a "Memorandum" to Marcus Rhoades in January of 1949 (1949 – APS, see page 191, Comfort, 1997), McClintock would repeat this striking pronouncement and declare that all mutations may be explained by her transposable elements. She further proposed possible biochemical and physical mechanisms to explain her concept of gene mutation. In her 1948 annual Carnegie research report, McClintock (1948b) declared that her new findings "cast doubt on the interpretation that postulates a 'true' gene mutation, that is, a chemical change in a gene molecule". She argued instead that phenotypic changes were due to reversible inhibition and gene modulation. Such a remarkable statement essentially challenged the underlying premise of Muller's recent (1946a) Nobel Prize. McClintock's ideas on this topic would crystallize and were aptly expressed in the opening sentence of the Discussion in her seminal 1953 Genetics publication when she wrote, "Mutations need not express changes in genes but may be the result of changes affecting the control of gene action."

In the early 1950's McClintock would publish two major papers on

[15] Luria had obtained a 27-page letter of June 1948 that McClintock (1948a) had sent to S.C. Stephens, a former McClintock colleague, detailing her findings and interpretations.

[16] The "intercepted" 27-page letter to Stephens that Luria based his seminar on and that motivated Muller's congratulatory note to McClintock was superseded only one month later (July 1948b) with McClintock's further challenge to Muller's assumptions of gene mutation when she wrote; "Assumption that mutations occurring frequently are chromosomal changes is more reasonable than an assumption of a change in a gene in a certain direction." (McClintock, 1948c - Odd note September 1948, APS, Box 9, Series V.).

transposable elements, mutable genes, and her concept of gene mutations (McClintock, 1950, 1951). These papers have become classics, being cited over 600 and 1000 times, respectively, in the Web of Science database, and would have a major impact on Muller's best friend and longtime Drosophila collaborator, Edgar Altenburg, at Rice University. Altenburg (1952a, 1952b, 1952c), who appeared to have been unaware of Muller's past communication with McClintock, wrote Muller on August 18 and 30 as well as on October 29, 1952, highlighting the significance of McClintock's findings and strongly encouraging Muller to study McClintock's papers. Altenburg would write Muller again on March 4, 1953, to repeat his previous suggestion and to give Muller a copy of his 25-page review manuscript on McClintock's research findings, a manuscript that had received McClintock's strong support/endorsement (Comfort, 2001). In this same letter Altenburg challenged Muller's two recently written chapters for a comprehensive genetics series edited by Alexander Hollaender. Altenburg stated to Muller, "In my opinion your Hollaender chapters suffer by lack of reference for Dr. McClintock's work. There are at least several places in your manuscript where it could have been profitably considered".

In his detailed 25-page review of McClintock's research on mutable loci in maize (January 1953), Altenburg concluded, "many of the mutants produced by X-rays undoubtedly represent position effects.[17] In this connection it is very significant that chromosome breakage produced by X-rays are in large measure in heterochromatin regions. Therefore, in X-ray treated material, it is not improbable that, in many cases at least, fragments of heterochromatin get inserted next to genes in other locations and suppress their phenotypic expression....". According to Altenburg's review, reverse mutations in Drosophila as well as maize, "are undoubtedly due in some cases at least to the release of normal genes from the suppressing effects of foreign heterochromatin adjacent to them, by deletion of the heterochromatin from a locus." This interpretation by Altenburg represented a unique challenge to Muller because his close colleague and collaborator (Altenburg) had apparently become convinced that Muller's interpretation was inadequate to account for the reverse mutation findings and needed to be modified and integrated into the McClintock framework, something that he probably knew would be very hard for Muller to accept (Altenburg, 1953a, 1953b).

Given that McClintock's findings on trans-positional mutator genes were highly relevant to the longstanding debate between Stadler and Muller over X-ray-induced gene mutations, one might have thought that Muller would have eagerly taken an interest in her challenging findings. A check of the Web of Science database on the key McClintock papers (1950, 1951, 1953) reveals that Muller never cited these papers. This lack of engagement with McClintock's findings was likely intentional, and suggests a strong element of strategic and tactical planning to avoid a direct conflict with McClintock.

In the previously mentioned 1948 letter to McClintock, Muller expressed his relief to have learned that her mutable genes were different from his proposed X-ray-induced gene-mutation model. At the time, McClintock emphatically disagreed with Muller's wishful and premature assumption and, ultimately, she would be proven correct. Over many years, McClintock's transposable elements (TEs) were shown to permeate organismal DNA (including Muller's Drosophila model) and to markedly affect mutation rates either in the absence or presence of exogenous agents such as ionizing radiation and chemical mutagens. Because TEs are powerful mutagenic factors that affect genomic instability, species have evolved highly diverse molecular strategies (e.g. RNA-induced posttranscriptional gene silencing to mediate the degradation of TE transcripts) to protect their respective genomes from the harmful activities of TEs. The fact that silencing of TEs profoundly affects background- and induced-mutation rates as well as cancer risks (Sturm et al., 2015) indicates that McClintock's model is superior to Muller's and therefore is a much better alternative (MacPhee, 1993; Sobels and Eeken, 1988; Velkov, 1999; Morales et al., 2015; Pesheva et al., 2005, 2008; Dimitrov et al., 2011). Likewise, in an integrated assessment of essential factors that are known to affect aging and lifespan, Sturm et al. (2015) concluded, "ageing is primarily caused by transposition–associated genomic instability."

Drosophila studies have now shown that X-rays can induce McClintock's mutator-gene system in a dose-dependent manner, with the upper part of the dose range overlapping with Muller (1927a) dose range (~1000–3500 r) (Handler and Gomez, 1997; Ratner et al., 2001). Furthermore, it was found that results from early Drosophila studies conducted by Timofeeff-Ressovsky et al. (1935) on radiation-induced mutations correlated amazingly well with results from studies conducted 66 years later by Ratner et al. (2001) on the induction of mobile genetic elements. This surprising correlation inspired Ratner's team (2001) to investigate a mechanism that may explain how mobile genetic elements could mediate X-ray-induced mutations. Other studies have shown that X-rays first activate mutator genes and then they, in turn, synergistically enhance the production of X-ray-induced mutations (Sobels and Eeken, 1981, 1987; Margulies et al., 1986, 1989; Balter et al., 1992; Zabanov et al., 1995; Ratner et al., 2001). Such results are particularly germane to the evaluation of the LNT-Single Hit model in that they strongly suggest interactions between the radiation dose, the transposable elements and the production of a range of genotoxic/mutational endpoints. Although the precise nature of such molecular and biophysical interactions still needs to be better defined, it is clear that McClintock's general concept has been strongly supported and that, as she predicted, there exists a mechanistic and sequential relationship between the radiating dose, the activation of TEs and, finally, the phenotypic display of a radiation-induced mutation.

In summary, the findings and interpretations of McClintock strongly challenged the Muller gene-mutation interpretation at multiple levels and in a manner that became progressively accepted by the research community for its scientific and technical accuracy and significance. Given the pristine reputation of McClintock within the genetics community for her scientific credibility, rigor, accuracy and intelligence, it appears that Muller chose not to challenge her alternative model for radiation-induced gene mutations, even when it conflicted directly with the argument on reverse mutations that he needed to defend his own model (Patterson and Muller, 1930).

During the summer of 1955 the NAS created the BEAR Committee, Genetics Panel. It was quite significant that neither McClintock nor anyone with her alternative perspective on gene mutation was invited to be a member of that panel.[18] A review of all transcripts, member reports and meeting summaries provided no evidence that the Genetics Panel discussed McClintock's alternative gene-mutation model. A review of the credentials of the geneticists on the panel revealed that

[17] In 1953 Peter Peterson, a maize researcher at the University of Iowa, reported the discovery of the mutable pale-green (pg) locus in strains of maize experimentally subjected to the atomic explosions at Bikini (Peterson, 1953, 1991). These findings supported the argument of McClintock/Altenburg.

[18] It should be noted that the Director of the Carnegie Institute, Milislav Demerec, McClintock's supervisor, was an invited member of the BEAR I Genetics Panel. In 1946, as mentioned in the Introduction of this paper, Demerec asked Ernst Caspari the key question of "what could be done to save the hit model?" following his striking findings. In addition, BF Kaufmann, the Assistant Director of the Carnegie Institute was also a member of the Genetics Panel. Calabrese (2015b) had earlier shown how Kaufmann misrepresented data that refuted a linear dose response in his oral presentation to the Genetics Panel. Kaufmann would replace Demerec as the Director of the Carnegie Institute in 1960. In a rather remarkable temporal coincidence, Caspari would spend 1947–1949 at the Carnegie Institute. Thus, three Drosophila geneticists associated with key aspects of the LNT model worked within the Department of Genetics at the Carnegie Institute with McClintock at the same time she was challenging Muller's gene mutation concept. It is not known whether and to what extent McClintock and these other individuals commented on this fundamental and very sensitive issue. It is also not known whether Demerec affected a possible appointment of McClintock to the Genetics Panel or whether he may have exerted pressure upon McClintock as she was challenging not only the basis of Muller's gene mutation model but also the fundamental underpinnings of the LNT model.

seven members (Beadle, Cotterman, Crow, Little, Sonneborn, Sturtevant, Wright) had never published a paper on mutations, the key focus of the Panel. Glass had published only two papers on mutations over two decades. Likewise Kaufmann had a weak record of publishing on mutations (4 in total). Essentially all the academic geneticists had received Rockefeller Foundation funding. That the Rockefeller Foundation/NAS would have invited so many geneticists with no experience in the area of mutations and dose responses to serve on this BEAR I Genetics Panel is a striking observation that has not yet been noted in the literature. Yet, the Panel was portrayed as a team of all-stars with a high level of expertise and experience in the induction of mutations by radiation and in their dose-responses. Furthermore, the Panel actually served to validate and promote Muller's gene mutation model, which contrasted significantly with McClintock's gene mutation alternative. To make matters even more untenable, the evaluation by the Genetics Panel was undertaken in the presence of Muller, whose dominating personality, ideological conviction to his model and profound and obvious conflict of interest should have disqualified him as member of the Panel.

It's curious that two years later the NAS would constructively engage or, depending on your point of view, distract McClintock on a more than two-decade international involvement on the evolution of maize (Comfort, 2001). The activity demanded prolonged periods in South America and an intense amount of her time and energy, effectively deflecting her from valuable research needed to advance her model and debate Muller before his death in 1967. No matter how valid and important was McClintock's challenge to Muller's model, it would undoubtedly wane without her attention.

On the other hand, Muller would soon be confronted with a serious question, this time involving the LNT Single-hit Hypothesis. In a highlighted paper in Science on December 19, 1958, William L. Russell (Oakridge National Laboratory) reported the discovery of radiation-induced, dose-rate effects in the spermatogonia and oocytes of mice. These findings were deemed so significant and challenging to the LNT concept that Muller redirected his laboratory to the assessment of dose rates on radiation-induced mutations in Drosophila. Russell's findings would significantly challenge the prevailing mantra that declared radiation damage to be cumulative, irreversible and linear in its dose response. In contrast to the mantra, Russell showed that radiation-induced mutations in mouse oocytes yielded an unambiguous threshold response. A detailed historical assessment of the Russell-Muller dose-rate debate and its impact on radiation risk assessment was recently published (Calabrese, 2017a, 2017b).

Why did McClintock's alternative gene-mutation model fail to gain traction and why has it remained essentially unknown to the scientific community, never taking part in the cancer risk-assessment debate? Table 2 compares Muller and McClintock gene-mutation models for trans-generational phenotypic changes, and identifies reasons why the McClintock gene-mutation model was not adopted. Reasons for failure may be separated into two groups. The first group describes McClintock's failures and explains how they prevented her success (see Table 2, numbers 1–9). The second group lists the factors that enhanced the acceptance of Muller's model (see Table 2, numbers 10–14). Although the factors were many that contributed to Muller's success and McClintock's failure, in the end it boiled down to leadership. Muller grabbed for the leadership mantle while McClintock did not. Differences in their personal histories, goals and priorities, for example, probably all contributed to some extent to the leadership differences between them. Nonetheless, McClintock's scientific visibility quotient, in terms of published papers and conference presentations needed to advance her gene mutation model as a superior alternative, was so low compared to Muller's that her alternative model failed to become part of the risk assessment debate for the past nearly 70 years. In fact, her gene mutation theory/model was not mentioned once in the 250,000 pages of the official OSHA record of regulation hearings for major carcinogens during the 1978–1980 time period (OSHA, 1980). Despite this striking failure, the transposon rediscovery and "revival" of the past 40 years have had profound biological, biomedical and clinical significance, and yet it remains decoupled from the cancer risk assessment process.

7. The modern ERA

Numerous studies have been published depicting the influence that ionizing radiation has had on the spectrum of mutational types of damage, ranging from base substitutions to large deletions and including a broad range of biological treatment models and a number of doses and dose rates (Webber and de Serres, 1965; De Serres et al., 1967; Thacker, 1986, 1992; Thacker et al., 1990; De Serres, 1991; Fossett et al., 1994; Nelson et al., 1994, 1995; Park et al., 1995; Yamada et al., 1996; Colussi and Lohman, 1997; Colussi et al., 1998; Nohmi et al., 1999; Schwartz et al., 2000; Mognato et al., 2001; Furuno-Fukushi et al., 2003; Liu et al., 2003; Nakamura et al., 2005; Sudprasert et al., 2006; Toyokuni et al., 2009; Okudaira et al., 2010; Russell and Hunsicker, 2012; Asakawa et al., 2013).[19] Reliable general patterns of response have emerged indicating that the type of damage is highly dose and dose-rate dependent. Low doses/dose-rates are generally associated with different types and proportions of lesions than occur at high doses/dose rates. At low doses/dose-rates, various base changes (i.e., transversions, transitions, and tandem based substitutions), frameshifts, and small/modest sized deletions/insertions may occur. As the dose/dose rate increases there is a switch to progressively larger types of genetic damage, ranging from partial to complete gene to multi-gene lesions (e.g., deletions). In the case of mature spermatozoa, the proportion of large deletions is about 4–5 fold greater than seen in the spermatogonia stage, possibly due to DNA repair (Ward and Alexander, 1957; Russell et al., 1998; Russell, 2004). The results also imply that each type of genetic damage most likely has a unique/separate origin/mechanism. Such circumstances led Schwartz et al. (2000) to conclude: "risk from low-dose radiation exposure cannot be easily extrapolated from high-dose effects." Of particular note is that about 60 years after the report of Muller (1928a), Southern blot analysis of nucleotide sequence could not differentiate damage below 50 base pairs. The use of Southern blot therefore led to a practical definition of a "point" mutation (i.e., genetic damage $\leq$ 50 bp could include small deletions, frameshift mutations and a variety of base changes) (Grosovsky et al., 1988). Regardless of the lack of capacity in the mid-1970s to late 1980s to resolve the spectrum of point mutations, the dose-dependence of genotoxic endpoints was readily apparent.

In 1927 the information available on X-ray-induced genetic damage was literally a "black box" consisting of poor resolution of chromosome structure, inadequate cytogenetic capabilities, and a rudimentary understanding of the range of genetic damage. This knowledge void existed within the context of radiation exposures that at times included or bordered on induced sterility. In retrospect, what Muller called a "point" mutation was in fact a euphemism for an ambiguous type and imprecise amount of genetic damage rather than for a known discrete biochemical lesion with precise physical characteristics. Despite considerable uncertainty, massive interpretative overreach and apparent errors, Muller would convince the genetics community and regulatory agencies to accept his X-ray-induced gene mutation and dose-response

[19] In his 1927 paper Muller pointed out that X-ray-induced genetic damage was likely to be dose dependent. He stated that X-ray-induced chromosomal aberrations/rearrangements would be "less likely than point mutations to depend on single quanta." (Note that he did not define or reference "quanta". He then argued, "a re-examination of the effect of different dosages must therefore be carried out, in which different types of mutations are clearly distinguished from one another." (page 87, Column 1). When assessed for a broad range of X-ray dosages he argued that it might then be possible to determine whether low levels of background gamma radiation cause the type of "spontaneous" mutations observed in nature or without an experimental treatment. The Science paper therefore reveals that Muller was aware that his high-dose treatment had caused massive genetic damage. However, it is obvious that he was hoping that the dosing he used produced "point mutations".

Table 2
Models of Transgenerational Phenotypic Changes (TPCs).

The Muller Gene Mutation Model	The McClintock Gene Mutation Model
Change in the Chemical Nature of the Gene	Gene Regulation/mutator Gene Concept/transposition Elements
TPCs are induced by direct hits of ionizing radiation on a gene, inducing mutation; indirectly via a host of possible secondary reactions.	TGCs are principally or possibly fully mediated by transpositional elements (TEs) via cellular regulatory processes. TPCs could be induced by agents that activate transposition elements.

Fate of the McClintock Gene Mutation Model: Why It Failed to Gain Traction for Cancer Risk Assessment During the 1950s and 1960s

1. McClintock's transposon concept was very complex and experimentally difficult to adopt for most contemporary labs; it was also not readily understood and adopted by the scientific community.
2. The death of Stadler in 1954 hurt McClintock's chances of success.
3. McClintock failed to get her supervisor, Milisav Demerec, to become a strong promoter of her scientific vision. In fact, McClintock resigned from the Carnegie Institute in 1953 due to frustration with the poor management/leadership qualities of Demerec; Vannevar Bush, the President of the Carnegie Institute for Scientific Research, interceded to get her to reconsider and stay.
4. The failure of McClintock to get appointed to the BEAR I Genetics Panel represented a significant lost opportunity to educate the Panel of her model and modify and possibly block the acceptance of the Muller model and LNT and to promote her own gene mutation model.
5. Demerec and Kaufmann, leaders from the Carnegie Institute, failed to promote her model at the BEAR I, Genetic Panel meetings. This failure was continued over to BEAR 2 and 3.
6. McClintock did not have graduate students that could have carried on her research/policy agenda. This was a double-edged sword as the seminal discoveries of McClintock may have been missed had her efforts had been delegated to others.
7. Failure of Altenburg to publish his comprehensive review of McClintock's transposon findings and their potential role in explaining radiation-induced mutation.
8. McClintock failed to address how her model related to medical /X-ray and environmental risk assessment.
9. McClintock failed to build an effective support group/organization to test, evaluate and promote her gene mutation model.
10. Muller's model received broadened application when it served as the basis of the LNT-single hit model. The promotion of the LNT-single hit model by several Nobel Prize winners represented a critical development.
11. Muller's receiving the Nobel Prize was essential for external validation of his gene mutation model and its risk assessment applications.
12. The recommendation to support LNT based on the Muller mutation model by the Manhattan Project facilitated its adoption by US government agencies.
13. Muller's testing methods were simple, quick and inexpensive. The interpretations were straight forward and easily understandable.
14. Muller and his radiation geneticist colleagues, typically with Drosophila research backgrounds, were organized, communicated often and had a strategy to promote the Muller gene mutation model.

interpretations as matters of scientific fact.

Although Muller attempted to rebut Stadler's (1954) posthumous challenge in Science (Muller, 1956; Muller and Oster, 1957) by claiming that "point" mutations were induced at various loci based on their capacity to reverse mutate to normal phenotypes, Muller's reverse mutation criteria was by no means a "gold" standard for small genetic changes (Thacker and Cox, 1983).[20] They noted that mutations caused by positional-effects (gene expression effects mediated by large genetic rearrangements) and those induced by transposable effects could affect gene reversions without gene mutations. Of significance here is that the gene loci frequently studied in Drosophila may display unusually high frequencies of reverse mutations, a situation that corresponds in magnitude to their frequencies for forward mutations. Thacker and Cox (1983) suggested that this phenomenon might be a component of the insertion/deletion cycle of a transposable element (Green, 1967) as seen in the use of the white apricot loss of Drosophila (Green, 1961).

Another severe blow to Muller's argument occurred when Voss and Falk (1973) used Muller's own biological model (Drosophila lethals) to examine about 600,000 gametes and found no evidence of "true" reverse mutations. In an earlier study from the same laboratory (Lifschytz and Falk, 1968), discrete and well-defined small zones within the chromosomes of Drosophila were occasionally found to have locus and region specificity for X-ray-induced mutations. More precisely, a small region around the maroon-like locus on the X chromosome demonstrated areas of large deletions that extended beyond a single locus. These same researchers also reported that all X-ray-induced post-meiotic lethals were due to chromosomal aberrations, further refuting Muller's argument.

In some studies, X-ray-induced mutations were frequently shown to involve a single locus. However, even in these cases, being "contained" within a single locus can still involve an extensive area of induced damage. For example, the size of one Drosophila gene locus is approximately 20,000 DNA base pairs (McGinnis et al., 1980) and therefore is theoretically large enough for extensive intra-gene damage to produce a mutation. Furthermore, large rearrangements or inversions can sometimes be misclassified as single gene changes even though one breakpoint of the inversion is located inside the zone of one gene and the other outside (McGinnis et al., 1980).

Bedford and Dewey (2002) published a paper on the "real" mutation controversy during and after the Muller era. In their summary they concluded, "no convincing evidence for the production of so-called point mutations was ever found for plants." Since Muller "found evidence for reversion of some X-ray induced mutations with Drosophila, he held the view that point mutations were in fact produced. In light of more recent information others have suggested that reversibility is by no means proof of a point mutation." Muller's argument equating "point" and reverse mutations in Drosophila may have been the key reason (along with a truly dominating leadership role in the field) that his gene mutation hypothesis prevailed (Stadler, 1932) for so long. Eventually, however, his hypothesis progressively succumbed to the combined effects of methodological advancements, invalidating experimental evidence, and the eroding support of his scientific community.[21]

[20] In this "rebuttal" Muller (1956) was forced to acknowledge that substantial findings on Drosophila had revealed that a very large proportion of what he called "point" mutations were now clear-cut deficiencies and other structural changes, a conclusion indistinguishable from that of Stadler. More specifically, Muller (1956), page 136) wrote that "...there is no doubt that in X-rayed Drosophila also, at least when the irradiation is applied to condensed chromosome stages, such as those of spermatozoa, deficiencies as well as other demonstrable structural changes arise with much higher frequency, relative to changes that appear to involve but one gene, than they do without treatment." This statement clearly reflects the eroding of Muller (1927a) position concerning the similarity of X-ray-induced vs spontaneous mutations and his statement that a high proportion of the gene changes were point mutations. It appears that Muller had given up considerable ground in the argument but still tried to retain some "wiggle room" so as not to raise the "white" flag of full intellectual surrender.

[21] In 1978 Charlette Auerbach, whose career was positively redirected by Muller in 1938 at Edinburgh, addressed the debate between Stadler and Muller over whether Muller had produced real gene mutations in his Nobel Prize research. For one with very close ties to Muller, it was very telling that she concluded that Stadler had firm experimental evidence with maize that confirmed his assertions, while Muller's evidence was suggestive but less direct. Such an assessment speaks highly for the intellectual penetration of the Stadler position. Auerbach (1978) then came to the defense of Muller (without providing a reference) stating that recent molecular analyses had "confirmed Muller's conclusions" that X-rays can produce true mutations. However, Muller was claiming that he had induced large numbers of mutations (i.e., "the artificial transmutation of the gene") but at the doses he used, the vast majority (perhaps all) were heritable chromosome aberrations. Many years later Muller's former post-doctoral colleague William R. Lee would report that "molecular analysis of X-ray-induced mutations in the germ line of Drosophila shows that nearly all are the result of X-ray-induced strand breakage" (Byrne and Lee, 1989; Batzer et al., 1988). The radiation doses studied by Lee were 5 Gy, 10 Gy, and 30 Gy with the doses reflecting those used by Muller. The findings of Lee and his colleagues strongly supported the position of Stadler in his debate with Muller. Thus, at the doses Muller tested he induced chromosomal aberrations and not "massive" new

8. LNT single-HIT hypothesis formulation

The debate between Muller and Stadler was not simply about the mechanism of X-ray-induced mutations nor was it simply between Muller and Stadler. By the early 1940's, it had expanded significantly to include issues related to the linear dose-response and the single-hit models used to assess both genetic and cancer risks.[22] Furthermore, Stadler was not challenging only Muller but also other leading geneticists as well as a cadre of elite physicists, including the future Nobel Prize recipient Max Delbruck and other leading radiation geneticists (e.g., Timofeeff-Ressovsky).

Muller and Timofeeff-Ressovsky used X-ray-induced sex-linked recessive lethality in Drosophila to assess trans-generational phenotypic changes and, as a result, demonstrated a linear dose-response relationship. The fact that some somatic cells of Drosophila showed phenotypic trait reversal suggested the possibility of X-ray-induced gene mutations. Delbruck and others applied target theory to the linear dose-response results and, together with mathematical modeling by Zimmer (1941), indicated that only a single hit could yield a linear dose response (Fig. 1).

The major assumption of the LNT single-hit model is that low-dose effects are predictable from high-dose exposures. Such predictions based on this major assumption suggest that the damage produced at low doses is qualitatively the same as at high doses (i.e. same type of genotoxic damage and with similar spatial pattern distribution) and that quantitatively the amount of damage produced is directly proportionate to the dose.

Delbruck constructed a tentative "atomic physics" model of the gene, as inferred from the frequency of "point" mutations under varying physical conditions. The views of Delbruck were popularized and markedly advanced by the Nobel Prize-winning physicist Erwin Schrodinger (1944) in his book What is Life. The key features of the model were that the (1) gene is a molecule; (2) that a mutation of a gene is a result of the transition from one stable state to another; and (3) the effect was due to thermal agitation or the absorption of radiant energy (Stadler, 1954). The linear dose response and the constancy of mutation yield regardless of time (i.e., Delbruck's model negated the occurrence of dose rate) predicted that X-ray-induced mutations from single "hits". The constant proportionality of mutation yield to ionization, regardless of wavelength variation, indicated that the unit was a single ionization. The average size of a gene was estimated as well as a quantitative characterization of the volume within which the hits occurred. This estimate was used to account for the mutations reported. He deduced that the average gene was comprised of approximately 1000 atoms. Delbruck then estimated that 1.5 eV would be required to induce a mutation with this model. Since the energy released via a single ionization is about 30 eV (i.e. far more than the 1.5 eV), the ionizing radiation should be able to mutate essentially all genes regardless of their inherent stability.

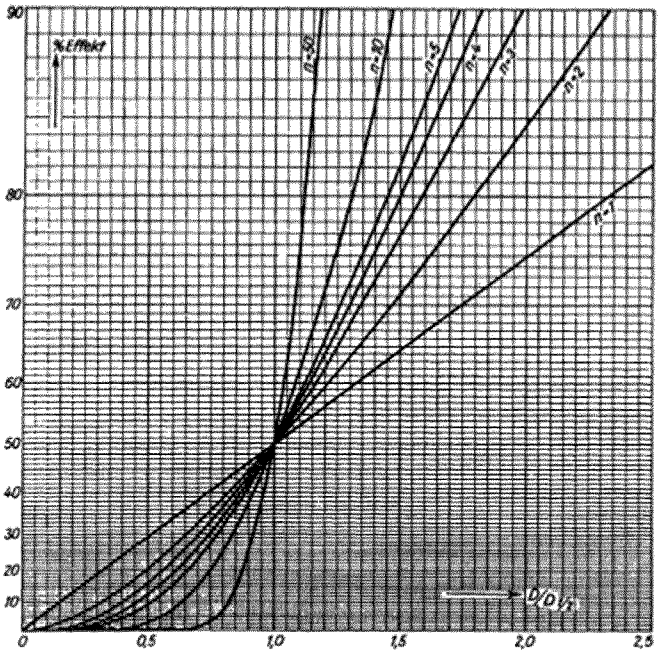

Fig. 1. Model dose-response curves, calculated theoretically for various number of "hits", n, on a single "target" assumed necessary to produce the effect. (From Zimmer (1941)).

9. Criticism of the LNT-single hit hypothesis

Stadler (1954) critiqued Delbruck's model and indicated "...it has been evident for many years that it (single hit theory) has no valid relationship to the experimental data from which it was derived." Furthermore, Stadler indicated that a non-gene alteration, such as a physical loss or rearrangement, represents a sound alternative to structurally altered genetic mutation and is strongly supported by evidence obtained in detailed assessments of X-ray-induced mutations. In concluding his critique, Stadler stated, "we have no basis for estimating the proportion of such extragenic mutations amongst the total of mutations observed and no grounds for assuming that this proportion is the same amongst the mutations observed under the various experiments."

The total dose and dose rate used during Muller's Nobel Prize research were extremely high. To provide a striking comparison, consider that Caspari and Stern (1948), who actually demonstrated an X-ray-induced threshold response in Drosophila, had to use a 50–60-thousand-fold lower dose rate than Muller did in his Drosophila studies. In addition to dose/dose rate concerns, the use of mature spermatozoa for cancer risk assessment was also problematic. Since mature haploid spermatozoa are more susceptible to X-ray-induced mutations than are cancer-forming diploid cells, it suggests that Muller's haploid spermatozoa are inappropriate for extrapolation to diploid somatic cells and, therefore, for cancer risk assessment. In contrast to spermatogonia and somatic cells, it was later discovered that DNA repair was absent in mature spermatozoa, offering a mechanistic explanation for the differential susceptibility to mutations. These concerns led the U.S. BEIR I Genetics Subcommittee (1972) to acknowledge errors made by the BEAR I (Anonymous, 1956) Genetics Panel on two issues: (1) using mutation data from mature spermatozoa to model risk of genetic damage and (2) considering dose rate as an irrelevant factor in cancer risk assessment (Calabrese, 2017b, 2017c).

It was not long after Muller's groundbreaking paper, according to Novitski (1976), before investigations began to counter Muller's gene-mutation hypothesis and reveal that chromosomal rearrangements were associated with sex-linked recessive lethals in Drosophila and that the proportion of these induced lethals increased with dose (Oliver, 1930; Demerec, 1937; Demerec and Fano, 1941). In fact, the general consensus was that lethal mutations were, as Novitski had noted, "a

(footnote continued)
"point mutations" as claimed.

[22] While the relationship between Muller and Stadler remains to be better clarified, there appears to have been a high degree of mutual respect (Muller, 1956). Of particular note is that from June 1948 until October 1949, Stadler was investigated by the FBI for concerns associated with linkages to communist groups within the US during the beginning of the Cold War. In fact, Stadler was denied a passport in June 1948 to attend the International Genetics Congress in Sweden. During this ordeal a number of leading geneticists came to the defense of Stadler. Based on obtained correspondence, Muller was strongly supportive of Stadler, showing remarkable leadership in this regard. While Stadler's answers to the FBI interrogatory were impressive in his defense so too were the actions of colleagues of very high stature such as Muller, Stern, Beadle, Demerec, and others. While Stadler was very grateful for the support of Muller, he and Muller would continue to scientifically debate on gene mutation until Stadler's death (May 1954) (Calabrese, 2017d).

Table 3
The LNT Single-Hit Model Timeline.

Date	Topic	Context
1927	X-Rays Induce Gene Mutations	Muller wins race to be first or did he?
1929–1931	Linear Dose Response	Follow up experiments by Muller's students support linear interpretation
1929	Improved Chromosome Cytology	Barbara McClintock develops markedly improved cytogenetic method for maize chromosomes.
1930	Muller Anticipates Challenges to Gene Mutation	Tried to prove gene mutation interpretations; used reverse gene mutation concept (Patterson and Muller, 1930).
1931	New Cytogenetic Methods Challenge Gene Mutation	Stadler and McClintock determine that X-rays induced heritable chromosome (not gene) changes.
1932	Stadler Challenges Muller's Gene Mutation Interpretation	Plenary address at the Sixth International Genetics Congress
1935	LNT Single-hit Model Formulation	Leading physicists/radiation geneticists create new model (Timofeeff-Ressovsky et al., 1935)
1936	Position Effect and Variegation/Mottling Effects	New mechanism could challenge parts of Muller gene mutation model.
1936–1960	Reverse Mutation (RM)	Significant research initiated to assess the frequency of RM induced by radiation and spontaneously. Results are mixed; they do not provide confirmation of Muller's gene mutation interpretation.
1938	McClintock Develops Procedure to Produce Mutations without X-Rays	The BFB method could induce a large amount of mutations in a targeted manner.
1939	Dose Rate and LNT Model	Muller's graduate student, Ray-Chaudhuri finds no evidence of dose-rate in mature Drosophila spermatogonia
1944	McClintock Discovers Transposable Genes	This discovery integrated the position effect and reverse mutations along with a theory of regulatory control over gene expression.
1943–1946	Manhattan Project Dose-Rate	Ernst Caspari study supports a threshold and dose-rate, challenges Ray-Chaudhuri findings (Caspari and Stern, 1948).
1948	McClintock's Alternative Gene Mutation Model	Transposon-based model leads to mutation without need for direct hit on gene; challenges Muller's model (Muller and McClintock correspondence).
1958	Dose-Rate Discovered in Mouse Model	Russell discovers dose-rate for X-ray induced mutation in mouse spermatogonia and oocytes.

result of chromosome breakage (Herskowitz, 1946; Lea and Catcheside, 1945; Haldane and Lea, 1947; Lea, 1946)". In time, follow up investigations were conducted that combined Muller-like doses with molecular mapping techniques to detect deletion breakpoints. These radiation exposure studies revealed a very high proportion of large deletions (Aaron, 1979; Fossett et al., 1994). These findings also revealed that ionizing radiation induced sex-linked recessive lethals can quantitatively predict the occurrence of induced deletions, including large deletion frequencies (Byrne and Lee, 1989; Fossett et al., 1994). These results strongly support the hypothesis of Stadler (1932, 1954) that most, if not all, of Muller's reported "mutations" were not gene mutations but rather chromosomal aberrations of various types and magnitude.

When the reverse mutation argument of Muller began to lose traction, Muller would shift the debate to the dose-rate experiments of Ray-Chaudhuri (1939, 1944). These experiments on dose rate gave rise to what may have been a type of desperate hope for Muller and other radiation geneticists who believed that the LNT single-hit model still had a defensible basis. This shift in debate may have been an underlying factor in Muller's less-than-critical evaluation of Ray-Chaudhuri's dissertation and, subsequently, in Muller's repeatedly deliberate mischaracterizations of Caspari's dose-rate study, which did not support the findings of Ray-Chaudhuri (Calabrese, 2011).[23] In his Nobel Prize lecture (December 12, 1946) Muller would use the Ray-Chaudhuri dissertation to affirm that there is "no escape from the conclusion that there is no threshold dose…". Muller's extreme dependency on the findings of Ray-Chaudhuri to refute the threshold model while simultaneously affirming the LNT single-hit dose-response model may have motivated his direct, powerful, coordinated and often deceitful attacks on the Caspari study (Calabrese, 2011, 2015a).

The LNT single-hit theory also failed to provide validated risk estimates across X-ray induced doses that produced qualitatively different types of gross chromosomal damage with the expectation of predicting responses to doses many orders of magnitude below those tested and for which analytical methods would not be available for almost a half century. The LNT single-hit hypothesis became progressively and seriously flawed in multiple reinforcing ways, over the next four decades following its formulation in the mid-1930s. While the stage should have been set for model revision, the radiation geneticist community, as lead by Muller, Stern and other colleagues, would do what was necessary to "save the hit model", and so they did (Calabrese, 2015a, 2017a).

10. Discussion

This paper assesses the historical origins of the LNT single-hit model and its reliance upon data obtained from studies (especially those of Muller) of X-ray-induced trans-generational changes in Drosophila phenotypes (Table 3). Also explored is the intellectual struggle that existed between Muller and Stadler over the authenticity of "gene" mutations as a mechanism to explain X-ray-induced trans-generational phenotypic changes at high doses. Painter developed his advanced cytogenetic staining method for Drosophila chromosomes (1934c) after Muller had already formulated his hypothesis on "true" gene mutations. McClintock and Painter's new cytogenetic methods revealed that at high doses, similar to those used in Muller's Nobel Prize research, chromosomal aberrations and/or rearrangements of various types would strongly predominate, if not exclusively, over "gene" mutations. In time, newer methods would progressively improve and refine the assessment of such gross chromosomal alterations, affirming the position of Stadler. Other arguments used by Muller, such as the reverse mutation phenomenon, were often not supported in multiple large-scale studies prior to (e.g., Lefeve, 1949, 1950; Neuffer, 1957) and after Muller's death (Voss and Falk, 1973). As scientific support for the gene mutation hypothesis began to erode, Muller and others of the radiation genetics community would shift their support behind the findings of Ray-Chaudhuri (1939, 1944) in support of an LNT interpretation. Although the substantial and integrated Caspari-Spencer follow-up experiments (Caspari and Stern, 1948; Spencer and Stern, 1948) challenged the data of Ray-Chaudhuri, major efforts were made to discredit these threshold (i.e., Caspari and Stern, 1948) supportive findings by Muller, Stern and other members of the radiation geneticist community

[23] The acceptance of the Caspari data would hinge on the validity of his control group data. Curt Stern contacted Muller at Amherst College late in 1946 and in 1947 to obtain his findings since Muller had amassed voluminous data on spontaneous mutation in Drosophila in an attempt to address the many scientific challenges of Stadler. As a result of this situation, the Muller data fully supported the Caspari position and his claims that X-ray induced mutation display both dose-rate and a threshold response. This was of fundamental importance as it refuted the LNT Single-hit hypothesis. See Calabrese (2011, 2015a) for detailed discussions. Thus, it is highly ironic that Muller's research to refute Stadler led to a confirmation of the Caspari findings that challenged the LNT Single-hit model.

(Calabrese, 2011, 2015a).

Muller and his colleagues were promoting the LNT single-hit model in the late 1940s and subsequent decades. However, had the cytogenetic advances of Painter (1934c) been available to Muller in 1927 (as McClintock's had become available to Stadler in, 1931), it is likely that Muller may have been more cautious in concluding that he had induced gene mutations. Nevertheless, Muller was clearly involved in a race to be the first to report that X-rays could induce gene mutations. Given all the publicity that Muller received, one can imagine that it would have been difficult for him to do anything but try to defend his interpretation.[24] Likewise, had this information been available prior to the formation of the LNT Single-hit model, this model may never have seen the light of day.

The findings herein complement a substantial series of papers on the history of LNT that started with the Manhattan Project (Calabrese, 2015a, 2015b, 2017a). These papers documented an era post-World War II that was dominated by strong ideological leadership in the community of radiation geneticists who managed to undermine the normal scientific practices of evaluation and review. During the period from 1946 to 1956, these leaders essentially directed a movement to promote and ensure the adoption of the LNT Single-hit model by regulatory agencies. It is interesting, however, that such widespread ideological behaviors were not evident prior to this period even though the same individuals dominated the field of traditional genetics. So then, what happened to change the traditionally accepted scientific practices of rigorous research, honest evaluation and unbiased debate to an ideologically driven ends-justify-the-means process entailing deceit, misdirection and harmful consequences?

The discovery of X-ray-induced mutation and its role in causing birth defects and cancer deaths stoked the fears of a public wary over nuclear holocaust in the immediate post-WW II era of the Cold War. X-rays used in newly developed medical techniques to diagnose and treat diseases as well as the possibility of significant human exposures to radioactive fallout from atomic bomb testing only heightened these fears. Society was ignorant, alarmed and fearful of radiation exposure and urgently demanded new policies from the federal government that could provide an assurance of safety and assuage public anxieties. The sense of urgency was palpable and driven largely by fear rather than the slow, deliberate and objective processes that are classic prerequisites to research, evaluation and debate. If not careful, however, this fear-driven sense of urgency could affect and skew the decision-making process of conscientious humans who are eager and feel pressured to quickly produce definitive results that protect the public and assuage anxieties while simultaneously satisfying their own needs as well as those of their superiors in the public and private sectors. A hurried and harried approach is antithetical to both good science and good management (decision-making) as is the failure to keep the functions and structures of science and management discrete and separate. Thus, keeping the science of risk assessment, for example, independent and separate from the human decision-making of risk management, which may be influenced by a fear-driven sense of urgency or other personal factors, would be necessary to avoid harmful and unintended consequences to society.

This potentially harmful mingling of science and management may well have been partially motivated by funding self-interest as well as a harried sense of urgency to fast-track a radiation health safety standard. The correspondence between members of the NAS BEAR Genetics Panels has shown that the members were not averse to exaggerating public health concerns in exchange for financial support for their research (Calabrese, 2014). Scientific self-interest has typically been attributed to scientists funded by industry, but the exchanges of letters among panelists make clear that they would deceive the scientific community and the public to obtain research funding. Likewise, their own words have been used to document that major funding groups—like the Rockefeller Foundation—(especially of the 1930–1960s) would manipulate these same panelists toward their scientific and policy interests (NAS/NRC, 1956 - see Weaver, page 35), an example of leveraging scientific funding that crossed ethical boundaries.

Lewis Stadler and others challenged Muller's gene mutation hypothesis at a time when it was sweeping the scientific and policy communities. As the data-driven challenges increased in strength over time, they began to weaken key aspects of Muller's high-dose gene-mutation model. This debate was protracted and profoundly significant, but finally reached a turning point with the findings of Caspari as they had the capacity to seriously undermine the adoption of the LNT Single-hit model. At this point, the tug-of-war between science and policy shifted and became an ideologically directed process led by scientists of great reputation, such as Stern and Muller, whose main goal was to ensure that the LNT Single-hit model would be adopted for cancer risk assessment. This story is now well documented (Calabrese, 2011, 2015a, 2015b).

The scientific foundations of the LNT single-hit model could not have been explored properly or completely without also examining the Nobel Prize research of Muller and the lifetime he spent defending it. As a result of this assessment, Muller's principal achievement may now be viewed from a slightly different perspective. Multiple leading geneticists (e.g. Timofeeff-Ressovsky, 1929; Crow and Abrahamson, 1997) of his era have claimed that Muller's importance was not so much that he was the first to produce gene mutations but rather that he showed how to produce copious mutations quickly (Campos, 2015). However, Muller's actions were focused almost entirely on the first question because he knew that this was the fundamental biological issue. How should one deal with the fact that 90-years of research have revealed that Muller did not produce the "gene" mutations that he had claimed in his 1927 Science article? Despite the historical significance given to both "gene" mutations and the LNT Single-hit model, the genetics field has remained essentially silent. Perhaps it is afraid to evaluate important aspects of Muller's story because he was an historical icon who had a major role in creating the field and establishing its centrality in biology. In fact, two esteemed geneticists and close allies with Muller had affirmed that Stadler was indeed correct in his claim that Muller produced mostly chromosomal deletions and not specific "gene" mutations (Crow and Abrahamson, 1997). In a review of the history of cytogenetics and genotoxicity, Garcia-Sagredi (2008) offered what seems to be an inspired Stadler-like remaking of the Muller discovery. He stated, "Muller (1927a) discovered that X-rays could cause mutations in Drosophila; specifically, that X-rays were able to produce rearrangements in the linear order of genes (for which he was awarded the Nobel Prize…"). However, rearrangement of the linear order of genes was not the goal Muller sought; it was the production of gene mutations. In the end, the greatest achievement of Muller was that he transformed the field of genetics with his exuberant, yet, incorrect claims. He inspired many laboratories to study radiation-induced mutations and was pivotal in influencing Charlotte Auerbach to assess chemical-induced mutagenicity, which opened up still another important new research area. He also awakened the scientific community and public to health concerns with excessive and unshielded radiation. Further, Muller's reporting of his mutation discovery in 1927 would inspire the creation of the Genetics Society of America during the December 1931 New Orleans meeting of the American Society of Zoologists (when the officers of the new Society were selected for 1932). The new society would play an important role in organizing the 6th International Genetics Congress at Cornell, an event that had highlighted Stadler's Plenary Address challenge to Muller's gene mutation claim. Muller did have an amazing impact on the field and society, but his 1927 paper should have an asterisk next to it.

[24] By not "winning" the race to first report X-ray-induced mutation, it provided Stadler with the opportunity to be more open to other possible data interpretations. Thus, even though his reports of X-ray-induced mutations in barley and maize seemed to fulfill all the standard features of gene mutations, Stadler would abandon this position after the cytogenetic findings of McClintock in the summer of 1931.

11. Conclusion

1. The LNT Single-hit model was based on the assumption that Muller had induced the "artificial transmutation of the gene" by X-ray treatment of mature Drosophila spermatozoa.
2. Several years after Muller had created the LNT model (i.e., called Proportionality Rule), it was afforded an explanatory mechanism via target theory and integrated into the Single-hit model.
3. Research by Lewis Stadler challenged Muller's basic conclusion that he had observed X-ray-induced gene mutations. This challenge was based on cytogenetic advances and the evaluations of Barbara McClintock concerning the effects of X-rays on corn. These findings suggested that Muller had predominantly, if not exclusively, induced heritable chromosomal (not gene) mutations, challenging the significance of his findings on heritable mutations.
4. Numerous subsequent studies in multiple biological models, including Drosophila, strongly supported Stadler's interpretation. Muller's claim that he had demonstrated the "artificial transmutation of the gene" and greatly enhanced the production of gene mutations were gross overstatements at best, which profoundly misled the scientific community and policy-making in public health areas. The LNT Single-hit model was not supported by the very biological data upon which it was based.
5. In a remarkable reversal of scientific support, leaders of the radiation genetics community placed considerable reliance on the findings of Ray-Chaudhuri (1939, 1944), which supported LNT.
6. Since subsequent integrative studies by Caspari-Spencer did not support the LNT study conclusion of Ray-Chaudhuri, but rather that of a threshold, the Caspari-Spencer studies became the object of unfounded and deceptive criticisms by Muller, Stern and other leaders of the radiation genetics community in an attempt to preserve the LNT Single-hit model.
7. The findings presented herein reconstruct the early development of the LNT Single-hit model up to the post Manhattan Project/modern cancer risk assessment era, and complement the substantial, previously published assessments on the history of LNT starting with the Manhattan Project.
8. These integrated findings lead to the conclusion that the LNT Single-hit model should have been neither recommended by the BEAR I Genetics Panel nor adopted for cancer risk assessment, as was the case in the U.S. and most countries.

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