[7] Davidson and Bogert, forthcoming publication.

[8] Davidson and Bogert, forthcoming publication.

[9] Behrend, *Ann. Chem.*, **245**, 213 (1888); Behrend and Ernert, *Ibid.*, **258**, 347 (1890).

[10] Angeli, *Atti. accad. Lincei*, [5] **3**, II, 72 (1894).

[11] Johnson, Baudisch, and Hoffmann, loc. cit.

[12] Levene, Bass, and Simms, *J. Biol. Chem.*, **70**, 229 (1926).

[13] Behrend and Roosen, *Ann. Chem.*, **251**, 235 (1889).

[14] Behrend and Osten, *Ann. Chem.*, **343**, 133 (1905); Behrend and Beer, *Ibid.*, **362**, 115 (1908).

[15] Levene, *J. Biol. Chem.*, **63**, 653 (1925).

[16] Behrend and Koch, *Ann. Chem.*, **315**, 246 (1901); Behrend and von Vogel, *Ibid.*, 259 (1901).

[17] This reaction suggests a mechanism for the formation of 5-phenylhydrazinouracil from the action of phenylhydrazine on 5,5-dibromo-6-hydroxy-5,6-dihydrouracil which was observed by Levene: namely, the formation of uracil-5-azobenzene (much as given above) followed by its reduction to the hydrazo compound by the excess of phenylhydrazine present.

SOME EFFECTS OF HIGH TEMPERATURE ON POLYPLOIDY AND OTHER VARIATIONS IN MAIZE[1]

BY L. F. RANDOLPH

UNITED STATES DEPARTMENT OF AGRICULTURE AND CORNELL UNIVERSITY

Communicated February 1, 1932

A renewed interest in the study of variations followed the recent discovery that profound changes in the heredity of organisms may be induced by x-radiation. Intensive studies in this field have demonstrated effects, some of which appear to be individual gene mutations modifying very slightly or not at all the organization of the chromosome as a morphological unit; others are known definitely to involve groups of genes and are exhibited not infrequently as visible structural changes in the architecture of the chromosome. These latter chromosomal variations have led to a better understanding of the probable mode of origin of morphologically unlike chromosomes in related species.

Variations of still another kind occur in nature and involve the duplication or loss of entire chromosome sets. From such variations have originated the polyploid series of multiple chromosome numbers, which are to be found very generally among related species distributed throughout the plant kingdom. The study of this class of variations has progressed slowly because of their infrequent occurrence under natural conditions. A technique for the production of new forms with increased or reduced numbers of chromosome sets would make possible the development of new

and improved varieties through the removal of incompatibilities of the type due to differences in chromosome number, and would provide much favorable material for other cytogenetic investigations of species relationships.

Numerous attempts have been made to induce polyploidy, and in certain cases a measure of success has been attained. Noteworthy are the classical experiments of the Marchals with mosses and of Winkler with Solanum. These and other more recent investigations have demonstrated the possibility of inducing polyploidy in somatic tissues by wounding, grafting, etc.[2] Such methods are, however, restricted in their applicability chiefly to plants which may be propagated asexually. The search for a method of wider applicability has continued.

The effectiveness of abnormally high temperatures in producing tetraploid cells and sectors in somatic tissues, chiefly of root-tips, has been noted by workers interested primarily in determining the reaction of the chromosomes to various physical and chemical agents (Nemec,[3] Sakamura,[4] Lundegårdh[5]). Sakamura observed an increased frequency of tetraploid cells in root-tips of Pisum which had been treated with hot water at 40 degrees Centigrade. Similar results with somewhat higher temperatures were reported by Koshuchow[6] working with the root-tips of Cucumis and Zea. Irregular chromosome division and fragmentation resulting from high temperature treatments were observed by Lundegårdh[5] in Vicia roots.

The relation of high temperature to gene mutation has been studied in Drosophila by a number of investigators. Muller[7] compared the frequencies of lethal mutations in cultures of flies held at 19 degrees Centigrade and at 27 degrees Centigrade, and noted a slightly higher rate in the warmer series. The difference was considered to be significant although the cultures used in the experiments showed a high variability in mutation rate. Goldschmidt,[8] Jollos[9] and Rokizky[10] worked with much higher temperatures (37 degrees Centigrade) and reported markedly increased frequencies of induced mutations. Many of these were identical with previously known mutations and occurred repeatedly in their experiments.

In a series of high temperature experiments undertaken several years ago for the primary purpose of developing a technique for producing tetraploids in maize, preliminary tests were made to determine the effects of high temperatures on cell division in seedling root-tips. Seedlings with well developed seminal roots were immersed in hot water at from 40 to 45 degrees Centigrade for different periods ranging from 15 minutes to two hours, and were maintained thereafter under conditions favorable for growth. Root-tips were fixed at frequent intervals during the 24-hour period following treatment. Microscopical examination of the root meristems showed an almost complete cessation of growth immediately following treatment, as indicated by the absence of division figures. Division activity was

TABLE 1

KERNEL TYPES AND SEEDLING CHROMOSOME NUMBERS IN THE HIGH TEMPERATURE EXPERIMENTS

		KERNEL TYPES					SEEDLING CHROMOSOME NUMBERS				
		NORMALLY	DEFECTIVE KERNELS				4N				
EXPT.	SCHEDULED TREATMENT TEMPERATURE	DEVELOPED KERNELS SELF-COLORED	CHIMERAS*	PARTIALLY DEVELOPED ENDOSPERMS	GERMLESS	SCARRED ENDOSPERMS	TOTAL	2N	4N	CHIMERAS	8N	TOTAL
A	45	722	21	73	87	89	903	567	20	7	1	595
B	43	284	..	14	61	14	359	215	5		1	221
C	43	169	9	13	17	13	208	142	3	1	1	147
D	40	471	59	32	46	32	618	374	1	2		377
E	38	359	7	8	16	8	390	312		2		314
Total		2005	96	140	227	156	2478	1610	29	12	3	1654

* Only chimeras in which the area lacking the dominant character or group of characters involved more than one-sixteenth of the surface area of the endosperm were recorded.

slowly resumed, ordinarily after about one hour, with a complete recovery of the normal rate within two or three hours after treatment. Tetraploid sectors were found to be much more abundant in treated roots fixed 24 hours after treatment than in the untreated roots in the same experiments. In an experiment which may be cited as representative of a series of similar experiments, 104 roots treated at 45 degrees Centigrade for one hour were examined for tetraploidy and 15 were found to contain one or more sectors of $4n$ tissues. Among 84 untreated roots in the same experiment only one contained a $4n$ sector. Successive treatments applied to the roots at four hour intervals materially increased the number of $4n$ sectors in a given number of roots.

Having thus obtained an effective technique for inducing tetraploidy in root meristems, similar treatments were applied to the zygote and proembryo in an attempt to produce entire individuals with multiple chromosome numbers. This was accomplished in Experiments A and B (Table 1) by applying heat locally to the ear-shoots. The ear-shoot region of the plant was enclosed within a cylinder of wire mesh. The cylinder was plugged at the ends with cotton and surrounded by an electrical heating pad. The temperature of the ear-shoot was determined approximately by a thermometer placed against the side of the ear within the husks, the temperature being regulated by varying the amount of current passing through the heating element of the pad. A thermometer inserted in the

air space inside the cylinder recorded the air temperature about the ear. In Experiments C, D and E, the entire plants were placed for treatment in a room maintained at the desired temperature.

The method of applying the heat treatments consisted in maintaining an air temperature of 47–48 degrees Centigrade until the ear-shoot reached the scheduled treatment temperature (column 2, Table 1). After this temperature was reached it was maintained with only minor fluctuations for approximately an hour by adjusting the air temperature, following which it was allowed to revert to the normal field or greenhouse temperature. Since temperatures higher than 48 degrees often were injurious, refinements of the present method or entirely new methods of maintaining high temperatures within the tissues of the plant would have to be devised in order to test the effects of temperatures appreciably higher than 45 degrees Centigrade.

The ear-shoot treatments were applied in Experiments A, C and E during the 48-hour period beginning 27–30 hours after pollination, this being the interval during which the division of the zygote and the early divisions of the proembryo were observed to take place under the conditions of temperature which were maintained during the experiments. In Experiments B and D the treatments were begun earlier, at 22–24 hours after pollination, to include the period during which syngamy occurred. The treatments were repeated at regular four-hour intervals throughout the entire 48-hour period in the initial experiments. In later experiments treatments were confined to the period of rising temperature in the morning and early afternoon when cell-division activity was at a maximum, no treatments being given during the night when division activity was at a minimum. Three treatments usually were applied during each of the two or three days following that on which the pollinations were made. Thirty plants were treated in Experiment A, eight in Experiment C and 12 in each of the other three experiments.

The stocks used in the experiments were selected to provide material for a study of the mode of origin and other characteristics of tetraploids, and also to afford a means of identifying genetically other types of chromosomal alterations and to make possible the recognition of disturbances in the normal processes associated with syngamy. For Experiment A, and F_1 hybrid between two self-fertilized strains having colorless aleurone, yellow endosperm, and weak anthocyanin color in the seedling stage was pollinated by a homozygous sun-red, liguleless type with purple aleurone and colored scutellum $\left(\frac{A\ C\ r^w\ sc_x\ b-Lg\ pl-Y}{A\ C\ R^r\ Sc_x\ B-lg\ pl-y}\right)$. For Experiment B, a self-fertilized strain of a commercial variety was used and the plants were self-pollinated. For Experiment C, a self-fertilized strain with colorless aleurone, white endosperm, and lacking anthocyanin color in the

seedling stage was crossed with the same pollen parent that was used in Experiment A $\left(\frac{A\ C\ r^g\ sc_x\ b-Lg}{A\ C\ R^r\ Sc_x\ B-lg}\right)$. For Experiments D and E, a liguleless type lacking anthocyanin color in the seedling stage, heterozygous for the *C*-aleurone factor, and for shrunken, waxy, sugary, yellow endosperm was used as the pistillate parent. Supernumerary *B*-type chromosomes[11] had been introduced previously into this stock, and for these experiments plants with one or more, preferably an odd number, of *B*-type chromosomes were selected. A dilute sun-red, glossy seedling type with sugary, yellow endosperm was used as the pollen parent $\left(\frac{a\ \ b-lg\ \ R^g\ pl-Yy\ \ Gl_3-Susu\ Cc-Shsh-Wxwx}{A\ B-Lg\ r^r\ \ pl-YY\ gl_3-susu\ \ CC-ShSh-WxWx}\right)$. The writer is indebted to Professor R. G. Wiggans of Cornell University for the self-fertilized strains of commercial varieties, and to Dr. G. F. Sprague of the United States Department of Agriculture for the stock homozygous for scutellum color.

Effects of the heat treatments were noted as follows:

1. Doubling of entire chromosome sets.
2. Chromosomal deficiencies and translocations.
3. Direct morphological effects resulting in defective and scarred endosperms, germless grains, dwarfed and otherwise defective seedlings and mature plants.
4. Sterilization of ovules and pollen at the higher temperatures.
5. Deviations from the normal fertilization process, such as failure of syngamy, atypical syngamic unions and parthenogenesis.

The polyploids were detected by a microscopical examination of seedling radicles.[12] These determinations were verified in all cases by examining additional root-tip material, and in Experiment A were further supplemented by sporocyte and pollen examinations, tests of the behavior of the pollen in crosses with diploids, and by noting the segregation of characters in the selfed progeny. In Experiment A, 17 of the 20 plants having $4n$ roots were found by these later tests to be wholly tetraploid, the others being chimeras with $4n$ roots and $2n$ stem portions. The $4n$ chimeras listed in Table 1 were seedlings with radicles containing both $2n$ and $4n$ tissue, one-eighth or more being tetraploid.

The higher temperatures were more effective than the lower in producing tetraploids and aberrant kernel types, as shown in table 1. An apparent exception is Experiment D, in which a high frequency of endosperm chimeras, germless and otherwise defective kernels was associated with a relatively low treatment temperature and a low frequency of chromosome doubling. In this experiment, however, the ear-shoots received in addition to the heat treatments approximately 500 r-units of x-radiation 23 hours

after pollination. The high frequency of aberrant kernel types in this experiment probably was caused by the x-rays, since it has been shown that they produce alterations of this kind.[13] Attempts to induce chromosome doubling with x-rays have failed repeatedly in experiments with maize.

All of the endosperm chimeras in which the dominant character or characters were absent from one-sixteenth or more of the surface area of the endosperm and present in the remaining portion are included in table 1. Most of these probably were due to individual chromosome deficiencies, as demonstrated originally by Emerson[14] for untreated material and similarly interpreted by Stadler[15] for x-rayed material. Three hybrid grains with colorless aleurone and yellow endosperm and five non-liguleless seedlings lacking anthocyanine color, which occurred in Experiments A and C, also may be classed as chromosome deficiencies induced at a sufficiently early stage to produce entire endosperms and embryos with the deficiency. These seedling recessives were deficient in growth and partially sterile. The frequency of chromosome translocations, associated with partial sterility and altered synaptic behavior of the chromosomes in meiosis, was materially increased by the heat treatments. Among 139 individuals with normal vigor there were 11 semi-sterile plants and a smaller number which exhibited other larger percentages of sterility.

A study of the effects upon the fertilization process of heat treatments applied separately and in combination with x-radiation at the time of syngamy was undertaken in Experiments A and D, and the following variations were noted:

1. A new type of endosperm chimera, not previously reported in maize, originated from the cross *aa RR Cc—Shsh—Wxwx Yy Susu* × *AA rr CC—ShSh—WxWx YY susu*. Approximately one-half of the endosperm of this exceptional kernel was maternal in character, exhibiting the recessive colorless aleurone and waxy, non-yellow endosperm characters, and the dominant, non-sugary endosperm character contributed by the female parent. The remainder of the endosperm was hybrid in nature as indicated by colored aleurone, the complementary action of the three genes A, C and R being requisite to the production of colored aleurone. This portion of the grain also had non-waxy, yellow, non-sugary endosperm. This chimera cannot be interpreted as a single chromosome loss, but may be interpreted readily by assuming that one polar nucleus developed independently to form the maternal portion and the second polar nucleus fused with the second male nucleus to form the hybrid portion. Thus the colorless portion would be haploid and the colored portion diploid. The aleurone cells in the transition region between the colored and colorless portions were examined microscopically and those of the latter were found to be smaller than those of the former, thus adding further confirmation to this interpretation.

2. A diploid glossy seedling was produced in Experiment D from a colored starchy grain. If the glossy character alone were considered, this plant might be interpreted merely as a *Gl* deficiency, but the fact that it differed markedly from seedlings of this type in having normal vigor and in resembling in general appearance the male parental strain rather than the normal hybrid seedlings, suggested that it was a paternal diploid. If evidence from the selfed progeny of this plant, which has not yet reached maturity, becomes available, its mode of origin can be more definitely established.

3. A maternal diploid seedling also was produced in Experiment D. The parthenogenetic development of a haploid egg followed by an early doubling of its chromosomes is indicated as the most probable mode of origin of this exceptional seedling.

4. A maternal haploid appeared in Experiment B. Although this haploid and the three exceptional individuals already noted occurred among relatively few individuals in an experiment designed primarily to test the effects of heat treatments on the fertilization process, more data are required to establish a definite causal relation.[15] The evidence from the preceding three exceptional cases is somewhat better in that individuals of these types have not been reported previously in maize.

The effectiveness of heat treatments in producing gene mutations was studied in Experiment A. Progenies consisting of 25 or more individuals were grown from the self-pollinated ears of 113 plants treated in the initial stages of embryogeny. These plants were from six ears selected from among 12 fertile ears in this experiment as showing the most pronounced effects of the heat treatments. These progenies consisting of 3975 seedlings were critically examined for new characters. Induced recessive mutations, as well as characters originally present, should appear in these families. The chance that one or more individuals recessive for a character segregating as a simple mendelian recessive will appear in a random F_2 population of 25 individuals is 200 to 1. Two seedlings showing irregular chlorophyll striping similar to that observed in cases of maternal inheritance in maize were found in one family. In 15 of 33 families, all of which were from the same original cross, one or more glossy seedlings were found. The recurrence of this glossy mutant in approximately one-half of the F_2 progenies of one original cross strongly suggests that one parent was heterozygous for the character. The low frequency of occurrence of segregating mutants in these F_2 families was probably due to the fact that the stocks used in the experiments were from self-fertilized strains which were more nearly homozygous than the stocks ordinarily used in genetic studies in maize. Certainly there was no evidence from these experiments that high temperatures are effective in causing gene mutation, but tests on a larger scale might establish a causal relation.

The genetic changes induced by the high temperatures were expressed in modifications of, first, the processes associated with nuclear and cell division, and, second, the architecture of individual chromosomes. The former make possible among other things the experimental production of new polyploid strains, the duplication of the polyploid condition in existing species, the production of hybrids between species incompatible because of differences in chromosome number by equalizing the parental chromosome numbers before crossing, and the production of fertile tetraploid hybrids between species whose normal diploid hybrids are sterile. The latter provides a new technique for a study of the physical properties of individual chromosomes, and should aid materially in the search for the underlying causes of induced chromosomal variations. Since organisms in their natural environment are not infrequently subjected to temperatures as high as or higher than those employed in these experiments, it seems not unlikely that high temperatures have played an important part in the production of variations in nature.

[1] These experiments were aided by a grant from the NATIONAL RESEARCH COUNCIL, Committee on Effects of Radiation upon Living Organism.

[2] A résumé of this work was published by Ufer, Max, *Der Zuchter*, **1**, 225–230(1929).

[3] Němec, D., *Das Problem der Befruchtungs-vorgänge*, Berlin (1910).

[4] Sakamura, T., *J. Coll. Imp. Univ. Tokyo*, **39**, pp. 221 (1920).

[5] Lundegårdh, H., *Svensk. Bot. Tidskr.*, **8**, 161–180 (1914).

[6] Koshuchow, Z. A., *Zeitschr. für Erforschung der Nutzpflanzen*, **10**, 140–148 (1928).

[7] Muller, H. J., *Genetics*, **13**, 279–357 (1928).

[8] Goldschmidt, R., *Biol. Zentralbl.*, **49**, 437–448 (1929).

[9] Jollos, Victor, *Ibid.*, **50**, 541–554 (1930).

[10] Rokizky, P. Th., *Ibid.*, **50**, 554–566 (1930).

[11] Randolph, L. F., *Anat. Record*, **41**, 102 (1928).

[12] Subsequently it was discovered that the tetraploids and other members of the polyploid series could be identified by noting the size and distribution of the stomata in the seedling stage. The stomata of haploids were found to be definitely smaller and closer together than were those of diploids, while in the triploids, tetraploids and octoploids they were correspondingly larger and more widely separated. These differences may be detected at relatively low magnifications, as with a 20X hand lens.

[13] Stadler, L. J., these PROCEEDINGS, **16**, 714–720 (1928), and unpublished data.

[14] Emerson, R. A., *Amer. J. Bot.*, **8**, 411–424 (1921).

[15] Stadler, L. J., loc. cit.

[16] Haploidy in maize was first reported by L. J. Stadler, and the writer, in papers presented before Section O, Amer. Assoc. Adv. Sci., Des Moines, Iowa, 1929. (Unpublished.)