RELATION BETWEEN CAROTENOID CONTENT AND NUMBER OF GENES PER CELL IN DIPLOID AND TETRAPLOID CORN [1]

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INTRODUCTION

The evaluation of induced chromosome doubling as a method of plant breeding requires more information on the specific effects of chromosome doubling than is available at the present time. It is important that this information be procured from comparisons of related diploid and tetraploid strains of known origin and similar genetic constitution. Such strains have not been generally available because of the infrequency with which chromosome doubling occurs spontaneously and because of the lack of methods for the experimental production of autotetraploids generally applicable to crop plants. But with the development of the heat-treatment technique for the experimental doubling of chromosome numbers (*10*) [3] comparable diploid and tetraploid strains of corn (*Zea mays* L.) and other plants have been made available for study.

The effect of chromosome doubling on the carotenoid pigments in corn is of special interest because of the vitamin A potency of two of these pigments. The investigation reported in this paper was undertaken to determine the relation between carotenoid content, cell volume, and gene number in comparable strains of diploid and tetraploid corn and to determine the carotenoid content of examples of commercial varieties, inbred strains, and hybrids of ordinary diploid corn. A preliminary report of these studies has already been published (*12*).

The carotenoids of the corn kernel are located in the endosperm tissue, which is relatively homogeneous in cellular organization and is thus favorable material for a study of cell-volume relations. In corn of the ordinary diploid sort the chromosomes and genes are present in triplicate, while in the derived tetraploid there are six sets of chromosomes and genes. Since the character in question is definitely localized in the endosperm tissue, a favorable opportunity is presented for a study of the effect of gene number on the degree of development of the yellow pigment.

Yellow corn meal contains the carotenoid pigments beta-carotene and cryptoxanthin, precursors of vitamin A, and zeaxanthin, which

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[2] The carotenoid analyses were made by R. G. Hart in the dairy chemistry laboratory, New York State College of Agriculture.

[3] Italic numbers in parentheses refer to Literature Cited, p. 64.

has no relation to vitamin A activity (*7*). In animal experiments, Steenbock and Boutwell (*14*) demonstrated the vitamin A potency of yellow corn and the lack of potency of white corn. Later, Mangelsdorf and Fraps (*8*) and Johnson and Miller (*3, 4*) found a direct quantitative relationship between the amount of vitamin A potency and the number of dominant genes for yellow in comparable samples of corn.

Studies on the chemical composition of diploids and their autotetraploid derivatives have been made in relatively few plants. The relation between chromosome number and vitamin C content in apples was studied by Crane and Zilva (*1*), who found that triploid varieties had more of the vitamin than did the unrelated diploid varieties that were examined. Autotetraploid tomatoes also contain more vitamin C than the parental diploids, according to Sansome and Zilva (*13*). Chemical analyses of comparable diploid and autotetraploid strains of tomatoes and petunias were made by Kostoff and Axamitnaja (*5*), who reported that the tetraploid tomatoes had more nitrogen and water but less cellulose and ash than the parental diploid strain; but in *Petunia* the chemical composition of the diploid and tetraploid was essentially the same. The results with tomatoes should be interpreted in the light of the fact that tetrapoid tomatoes, because of their reduced fertility, have smaller fruits than the diploids.

PREPARATION OF MATERIALS

The strains of diploid and tetraploid yellow corn selected for comparison were derived from three inbred lines of pure yellow corn, Webber Dent 2312, Illinois A–2311, and Luces Favorite W36–1. The Webber Dent inbred was crossed with Illinois A, and the F_1 hybrid was crossed in turn with the Luces Favorite inbred. Chromosome doubling was induced in this three-way hybrid by the heat-treatment technique (*10*), and the resulting tetraploid plants, several in number, were mass-pollinated for two generations to provide adequate material for analysis. A comparable diploid strain was developed from the same source by mass-pollinating for two generations the diploid sister plants of the induced tetraploid individuals. The two strains were designated Diploid Yellow and Tetraploid Yellow, respectively. A second tetraploid yellow strain, designated Tetraploid Yellow B, was also analyzed for total carotenoid content. This strain originated from a cross between an inbred white flint corn and the Webber Dent 2312 inbred, followed by three generations of selective breeding for the yellow character. Comparable diploid and tetraploid strains of white corn, derived from inbred lines of white Argentine Flint and Spanish Flint and here designated Diploid White and Tetraploid White, were also analyzed for carotenoid content. In addition, separate analyses were made of a number of diploid inbred lines, including those from which the above-mentioned strains were derived.

In addition to these analyses of tetraploid and diploid corn, the carotenoids were determined in examples of various commercial varieties, inbred lines, and their hybrids, including a commercial double-cross hybrid grown extensively for grain and fodder in New York.[4]

[4] The hybrid, W29–3, and the parent lines, W36–1, W36–2, W36–3, and W36–4, were produced by Dr. R. G. Wiggans, of the Department of Plant Breeding, New York (Cornell) Agricultural Experiment Station, and were furnished by him for these studies.

The comparison of cell-volume relations in the endosperm tissue of the diploid and tetraploid corn was based upon a study of morphologically mature kernels selected from the pure yellow strains, which were analyzed for carotenoid content. Microtome sections were prepared in the usual manner from the desired portions of kernels that had been fixed in a weak Flemming's solution. To facilitate the preparation of the sections, the reserve starch in the endosperm was partly removed by soaking the seeds in water prior to fixation. Cell volumes were computed from measurements taken from camera-lucida sketches drawn at a magnification of 440 diameters.

ANALYTICAL METHOD

For the fractionation and determination of the carotenoids in the corn meal, the following simplified method, based upon the procedure of Kuhn and Brockmann (*6*), was developed. The meal was prepared from entire kernels, including the embryo and pericarp in addition to the endosperm. Since the endosperm comprised the bulk of the meal and the proportions of the different parts were very similar in the diploid and tetraploid stocks that were analyzed, the presence of the germ and pericarp was disregarded in comparing the relative amounts of carotenoid present in the samples. Samples were prepared for analysis by selecting at random 10 ears of corn from each strain. Corn meal was produced from the air-dried, shelled grain by grinding 50-gm. samples for 5 minutes in a Wiley mill equipped with a sieve of 1-mm. mesh. The meal was dried in a desiccator over phosphorus pentoxide (P_2O_5). Four to eight samples of 2.5 gm. each were thoroughly mixed with 50 ml. of anhydrous methyl alcohol (distilled from lime) and allowed to stand in glass-stoppered flasks for 18 hours. The methyl alcohol extract was filtered through sintered glass, the filter and precipitate were washed with small portions of methyl alcohol, and the volume was reduced by evaporation under reduced pressure and then made up to exactly 50 ml. with additional methyl alcohol. The total pigment in the methyl alcohol was determined in a photoelectric colorimeter with Corning glass filters 585 and 428. The construction, calibration, and use of this colorimeter has been described by Hand and Sharp (*1a*). The extract was saponified for 2 hours at 50° C. after 5 ml. of a 5-percent solution of potassium hydroxide (KOH) in methyl alcohol had been added. The solution was cooled, 6.0 ml. of water added, and the mixture shaken for 15 seconds in a separatory funnel. The active fraction, containing beta-carotene and cryptoxanthin, was extracted with from 4 to 8 successive 25-ml. portions of petroleum ether.[5] The petroleum ether extracts were combined and evaporated to exactly 50 ml. under reduced pressure, and the absorption coefficient was determined in the colorimeter. In a similar manner the inactive zeaxanthin was extracted with petroleum ether after the water content of the methyl alcohol had been increased to 40 percent, and the absorption coefficient in petroleum ether was determined.

Calculations of carotenoid were made from a calibration curve for the absorption coefficient of pure beta-carotene plotted against

[5] Separate determination of beta-carotene and cryptoxanthin can be made by extracting the beta-carotene from 99-percent methyl alcohol and the cryptoxanthin from 90-percent methyl alcohol, but were not made in these studies because of the relatively small amount of beta-carotene in corn meal.

milligrams of beta-carotene per liter of petroleum ether (fig. 1). In these calculations, it was assumed that cryptoxanthin and zeaxanthin had the same absorption as beta-carotene with glass filters 585 and 428 and that the light absorption was the same in methyl alcohol as in petroleum ether. Since these assumptions are not exactly correct, an error was introduced with respect to the absolute amounts of carotenoid, but not with respect to the relative values for the different

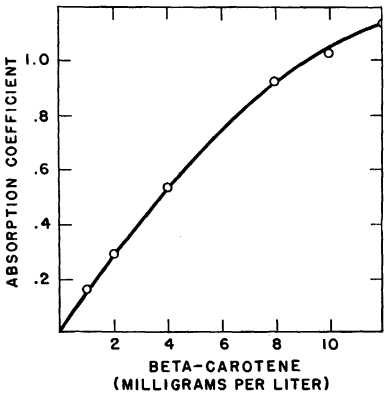


FIGURE 1.—Light absorption by beta-carotene in petroleum ether. Absorption coefficient, $\log_{10} \frac{I_o}{I}$ for 1 cm., filters 585 and 428.

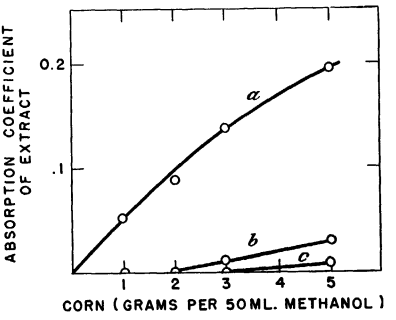


FIGURE 2.—Repeated extractions of corn samples by methanol: *a*, First extraction; *b*, second extraction; *c*, third extraction.

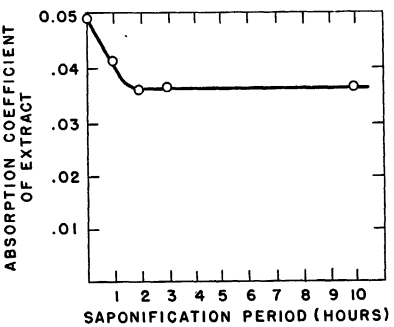


FIGURE 3.—Effect of saponification time on the extraction of carotenoids by petroleum ether from 85-percent methanol.

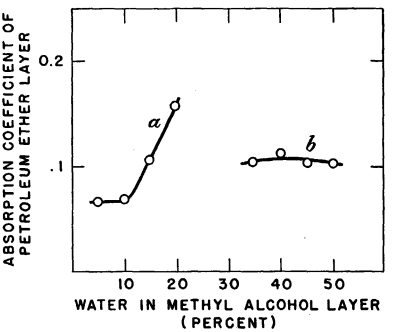


FIGURE 4.—Effect of water content on the fractionation of carotenoids: *a*, Active fraction; *b*, inactive fraction.

strains of corn. In practice the calculation was further simplified so that the number of milligrams of carotenoid per gram sample was obtained by multiplying the absorption coefficient by the factor 0.155. This approximate method neglects the deviation from linearity up to 5 mg. per liter (fig. 1). The chief advantage in using the factor is that the calculated values for carotenoid content can be readily changed to the experimental values for the absorption coefficients of 50-ml. extracts from 2.5-gm. samples of corn.

Experiments made in the elaboration of the method are summarized in the accompanying figures. In figure 2 it can be seen that practically all of the carotenoid is removed by a single extraction with 50 ml. of methyl alcohol if the sample is not larger than 2.5 gm. Figure 3 shows that the saponification of the xanthophyll esters is complete after 2 hours at 50° C. If the saponification is incomplete, the xanthophyll esters are extracted with the beta-carotene and cryptoxanthin and the values for the active fraction are erroneously high. If the saponification is carried on too long, e. g., for 10 hours, extraction of the zeaxanthin fraction by petroleum ether is very much more difficult. Figure 4 shows that a sharp separation of the active and inactive fractions is obtained with 10 percent of water in the methyl alcohol. If more water is used for the first extraction, some of the zeaxanthin is forced into the petroleum ether. The amount of water needed for the optimum extraction of the inactive fraction may vary from 30 to 50 percent.

Some idea of the accuracy of the method can be obtained from a series of 17 analyses of the same strain of corn for which the average deviation from the mean was only ± 4.2 percent. By reducing the number of steps in the procedure the chances for the loss or destruction of carotenoids were reduced. The results reported herein agree with typical values for the carotenoids in a sample of Italian corn reported by Kuhn and Grundmann (*7*) (beta-carotene, 0.0007 mg.; cryptoxanthin, 0.0046 mg.; and zeaxanthin, 0.0127 mg. per gram) but are considerably higher than those of Johnson and Miller (*3*, *4*).

RESULTS OF CAROTENOID ANALYSES

The results of the carotenoid analyses of diploid and related strains of yellow and white corn; of a number of commercial varieties of ordinary diploid corn, including examples of the more important kernel types, such as dent, flint, pop, and sweet corn; and of a limited number of diploid inbred lines and hybrids are shown in table 1. The total carotenoids in methyl alcohol, the active provitamin A fraction, containing carotene and cryptoxanthin, and the inactive zeaxanthin fraction are listed separately in the table. The sum of the active and inactive petroleum ether fractions does not in all cases equal the values obtained for the total carotenoids in methyl alcohol, presumably because of losses incurred during their saponification, transfer to petroleum ether, and final separation. Therefore, the values obtained from the original methyl alcohol extractions are considered to be a more reliable index of the relative amounts of total carotenoids present in the different kinds of corn that were analyzed.

The feeding experiments of Mangelsdorf and Fraps (*8*) demonstrated that the amount of vitamin A in corn meal was directly proportional to the number of dominant genes for yellow endosperm color present in the seed, the amount present being approximately in the ratio 3 : 2 : 1 for the *YYY*, *YYy*, and *Yyy* endosperm genotypes. White corn of the constitution *yyy* was found to have no vitamin A potency, a result which was in agreement with the earlier work of Steenbock and Boutwell (*14*) and Hauge and Trost (*2*). Results similar to those of Mangelsdorf and Fraps (*8*) were reported recently by Johnson and Miller (*3*) from spectrophotometric analyses.

TABLE 1.—*Carotenoid analyses of tetraploid and related diploid corn, diploid commercial varieties, inbred lines, and hybrids, in milligrams of pigment per gram of dry corn meal, each value being an average of four or more analyses*

[Standard errors are given for the total carotenoid values]

Corn sample	Total carotenoids in methyl alcohol	Sum of petroleum ether fraction	Beta-carotene and cryptoxanthin	Zeaxanthin
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
Diploid Yellow	0.0261±0.0005	0.0243	0.0097	0.0145
Tetraploid Yellow	.0366± .0010	.0314	.0122	.0192
Tetraploid Yellow B	.0389± .0006			
Diploid White	.0067± .0003	.0049	.0018	.0031
Tetraploid White	.0054± .0001	.0042	.0017	.0025
Commercial varieties:				
Leaming Yellow	.0266± .0001	.0267	.0068	.0199
Cornell 11	.0282± .0002	.0201	.0056	.0145
Alvords White Cap Dent	.0073± .0002	.0051	.0019	.0032
Argentine Yellow Flint	.0479± .0001	.0422	.0160	.0262
Queen Golden pop	.0352± .0002	.0325	.0117	.0208
White Rice pop	.0034± .0001	.0027	.0008	.0019
Yankee Cheat flour	.0044± .0002	.0028	.0008	.0020
Golden Bantam sweet	.0071± .0004	.0126	.0031	.0095
Inbred lines:				
Dutton Flint	.0144± .0001	.0113	.0035	.0078
Bloody Butcher	.0210± .0002	.0131	.0039	.0092
Illinois A–2311	.0257± .0001	.0183	.0047	.0136
Webber Dent 2312	.0327± .0001	.0237	.0065	.0172
Luces Favorite W36–1	.0270± .0001	.0271	.0108	.0163
Onondaga White W36–2	.0057± .0001	.0038	.0016	.0022
Cornell 11 W36–3	.0639± .0006	.0651	.0313	.0338
Bloody Butcher W36–4	.0476± .0003	.0421	.0178	.0243
Hybrids:				
W36–2 × W36–1	.0109± .0001	.0103	.0043	.0060
W36–3 × W36–4	.0705± .0001	.0709	.0328	.0381
W29–3	.0326± .0004	.0290	.0119	.0171

In the present study a comparison was made between the carotenoid content of pure yellow diploid corn carrying the three dominant genes, *YYY*, for yellow, and a derived tetraploid with the doubled number of genes, *YYYYYY*, for yellow. Increasing the number of genes for yellow from three to six increased the total carotenoid content from 0.0261 mg. per gram of dry meal in the diploid to 0.0366 mg. in the tetraploid (table 1), an increase of 40 percent. This was the percentage increase for the Tetraploid Yellow strain compared with the related Diploid Yellow strain. There was approximately the same percentage increase for both the active provitamin A fraction containing beta-carotene and cryptoxanthin and the inactive zeaxanthin fraction. That is, the increase in carotenoid pigment carried with it a proportional increase in vitamin A potency. The intensity of endosperm color was approximately the same in the diploid and tetraploid strains.

Although the 40-percent increase in carotenoid content is attributed to chromosome doubling, it is recognized that the growing of the Tetraploid Yellow and Diploid Yellow strains for two generations in order to obtain sufficient material for the carotenoid analyses provided a limited opportunity for the segregation of genes affecting carotenoid content to take place independently within each of these strains. Such genotypic changes might either diminish or accentuate the differences in carotenoid content caused by chromosome doubling. However, the inbred lines from which these strains originated, namely, Illinois A, Webber Dent, and Luces Favorite, did not differ markedly

in carotenoid content (table 1), and the opportunity for gene segregation to occur was reduced to a minimum by practicing mass pollination rather than selfing or sib-crossing individual plants.

A second tetraploid yellow strain that was analyzed, Tetraploid Yellow B, had a carotenoid content of 0.0389 ± 0.0006 mg. This strain was a third-generation selection for yellow from a cross between Webber Dent 2312 and a white Spanish Flint inbred and was still segregating for endosperm color. The carotenoid content of this impure yellow tetraploid strain was appreciably higher than that of its diploid yellow parent, Webber Dent 2312, which was 0.0327 ± 0.0001 mg. Ordinarily the admixture of white with yellow strains reduces carotenoid content. (See table 1 and the analyses of Mangelsdorf and Fraps (*8*), Johnson and Miller (*3*), and others.) The relatively high carotenoid content of the Tetraploid Yellow B strain is attributed to the effect of chromosome doubling.

The diploid inbred lines from which the pure Diploid Yellow and Tetraploid Yellow strains originated, namely, Webber Dent 2312, Illinois A–2311, and Luces Favorite W36–1, were somewhat different with respect to both endosperm color and carotenoid content. The Webber Dent inbred had the deepest endosperm color and also the highest carotenoid content, but the Luces Favorite inbred, which had the least endosperm color of the three, had a somewhat higher carotenoid content than the deeper yellow Illinois A line. The mean value for the three inbred lines was 0.0292 mg. per gram of dry meal, as compared with 0.0261 mg. for the diploid strain produced by intercrossing these lines.

The total carotenoid content of the Tetraploid Yellow strain was not twice as great as that of the related Diploid Yellow strain, as might have been expected since other workers have shown that in ordinary diploid corn the carotenoid content is directly proportional to the number of dominant genes for yellow endosperm. The carotenoid content per unit volume [6] in the tetraploid was 40 percent greater than in the diploid, but in terms of gene number per cell there was more than a twofold increase, as will be seen from the section on Cell-Volume Relations.

The tetraploid white-endosperm strain and the diploid from which it was derived contained appreciable amounts of carotenoids including beta-carotene and cryptoxanthin, which are precursors of vitamin A (table 1). In these strains, doubling the number of chromosomes and genes caused a decrease of 19 percent in carotenoid content. These white strains, which are designated Tetraploid White and Diploid White, originated from a cross between an inbred line of an early flint corn commonly known as Spanish Flint and an inbred line of white Argentine Flint corn. Other white-endosperm types of diploid corn, including White Rice popcorn, Yankee Cheat flour corn, and Onondaga White Dent corn, had a lower carotenoid content than the Diploid White corn (table 1). Alvord White Cap Dent, a yellow dent corn with a white crown, was also very low in total carotenoids.

In all of the tetraploid strains investigated, the weight of the individual kernels was approximately 50 percent greater than that of the

[6] Since approximate measurements showed no significant difference in the density of the diploid and tetraploid kernels, the carotenoid content for unit weight, i. e., per gram of dry meal, is also the carotenoid content per unit volume.

related diploid. This percentage increase in kernel size is characteristic of most strains of tetraploid corn (*11*).

The commercial varieties and inbred lines of diploid yellow corn that were analyzed exhibited a wide range of values for total carotenoid content, as indicated in table 1. The highest value (0.0639 mg. per gram) was obtained from an inbred strain of Cornell 11, and the lowest values were from Dutton Flint (0.0144 mg.) and Golden Bantam sweet corn (0.0071 mg.). The inbred line of Cornell 11 had more than four times as much carotenoid as the Dutton Flint.

It was noted that the yellow appearance of the grain was not a reliable criterion of carotenoid content. For most of the commercial varieties there was a positive correlation between intensity of endosperm color and the amount of carotenoids present in the meal. Golden Bantam sweet corn was a conspicuous exception. This is a rich yellow sort, but it has the lowest carotenoid content of any of the yellow kinds that were compared. However, Argentine Yellow Flint, a very deep orange-yellow type, had a higher carotenoid value than the deep-yellow dent varieties Cornell 11 and Leaming. Among the inbred lines there was no consistent relation between endosperm color and total carotenoids. A deep-yellow Dutton Flint inbred had only about half as much total carotenoid as a medium-yellow inbred line of Luces Favorite, the values for the two lines being 0.0144 mg. and 0.0270 mg., respectively. One inbred line of Bloody Butcher had a rather low carotenoid content, while a second one had more than twice as much. The highest carotenoid value that was obtained for any inbred line or commercial variety was that of a Cornell 11 inbred, which had 0.0639 mg. of carotenoid per gram of dry meal, as compared with 0.0282 mg. for the commercial Cornell 11 variety from which it originated. However, the color of the endosperm was essentially the same in the inbred and in the parent variety. These results, demonstrating a lack of correspondence between carotenoid content and intensity of endosperm color, are in agreement with the results recently reported by Johnson and Miller (*3*, *4*).

The commercial double-cross hybrid W29–3 and its parent lines were analyzed to determine the influence of hybridization on carotenoid content. In the production of this hybrid,[7] the parent inbred lines were combined as follows: (W36–3 × W36–4) × (W36–2 × W36–1), the first-named line being the seed parent of each hybrid combination. Since the endosperm tissue in which the carotenoids of the kernel are localized is triploid and originates from the combination of two sets of chromosomes and genes from the seed parent with one set from the pollen parent, the influence of the seed parent should be twice as great as that of the pollen parent in determining the carotenoid content of the hybrid, provided the genes affecting carotenoid content act in the cumulative manner described by Mangelsdorf and Fraps (*8*) for the yellow endosperm genes. The value for the single cross (W36–2 × W36–1), involving a white-endosperm type, Onondaga White Dent, and a medium-yellow type, Luces Favorite, is in close agreement with the expected value, being nearer that of the seed parent than that of the pollen parent. The other single cross (W36–3 × W36–4), which involved two deep-yellow endosperm types,

[7] WIGGANS, R. G. Unpublished data.

Cornell 11 and Bloody Butcher (the yellow endosperm of this variety is obscured by the presence of red pericarp color), had more total carotenoids than the seed parent. This is of special interest since the seed parent, Cornell 11, had the highest value of any of the diploid types that were analyzed. The increase in the hybrid may have been due to the complementary action of additional genes for yellow contributed by the two parents. The plausibility of this explanation is strengthened by the recent discovery that yellow endosperm color in maize is dependent on the interaction of the dominant allelomorphs of two or more genes (*9*). The double-cross hybrid W29–3 had a somewhat lower carotenoid content than the expected value.

The extreme variation in the carotenoid content of inbred lines of yellow corn, including both the active provitamin A fraction and the inactive fraction, emphasizes the importance of carotenoid determination to evaluate the feeding quality of these strains and their hybrids. The carotenoid content of the Cornell 11 inbred line, which had the highest carotenoid value of the five yellow inbred lines that were analyzed, was more than four times as great as that of the Dutton Flint inbred, which had the lowest value of these five lines. Since endosperm color is not a reliable criterion of carotenoid content, except within very broad limits, chemical analyses or biological tests with animals are necessary to determine at all accurately the carotenoid value of a given sample of yellow corn. Furthermore, the results obtained from the analyses of strains of white corn indicate that some of them, as for example, Diploid White, possess sufficient provitamin A carotenoids to be detected by animal-assay experiments. However, the animal experiments that have been performed (*2*, *8*, *14*) have failed to demonstrate any significant vitamin A potency of white corn.

CELL-VOLUME RELATIONS

Chromosome doubling ordinarily results in an increase in cell size commensurate with the increase in nuclear volume caused by the presence in the nucleus of the double number of chromosomes. Autopolyploids of recent origin invariably have larger pollen grains and larger stomata than the forms with lower chromosome number from which they originated, but not so much is known about cell-volume relations in many other parts of the plant, as, for example, in the endosperm. Since the endosperm of the corn kernel is a simple tissue made up of relatively homogeneous cell components, it is favorable material for a study of the influence of chromosome doubling on cell volume.

At the present time very little is known about the influence of the changed conditions of cell volume and gene number per cell upon the action of specific genes following chromosome doubling. In diploid organisms certain genes exhibit differential quantitative action, while others do not, under conditions in which cell-volume relations remain relatively constant. In autotetraploids both cell size and gene number per cell are greater than in the parental diploid, thus creating changed conditions under which the action of the genes may differ from their known action in the diploid organisms. An investigation of cell-volume relations in diploid and tetraploid corn was undertaken to determine the extent to which the concentration of the genes for yellow

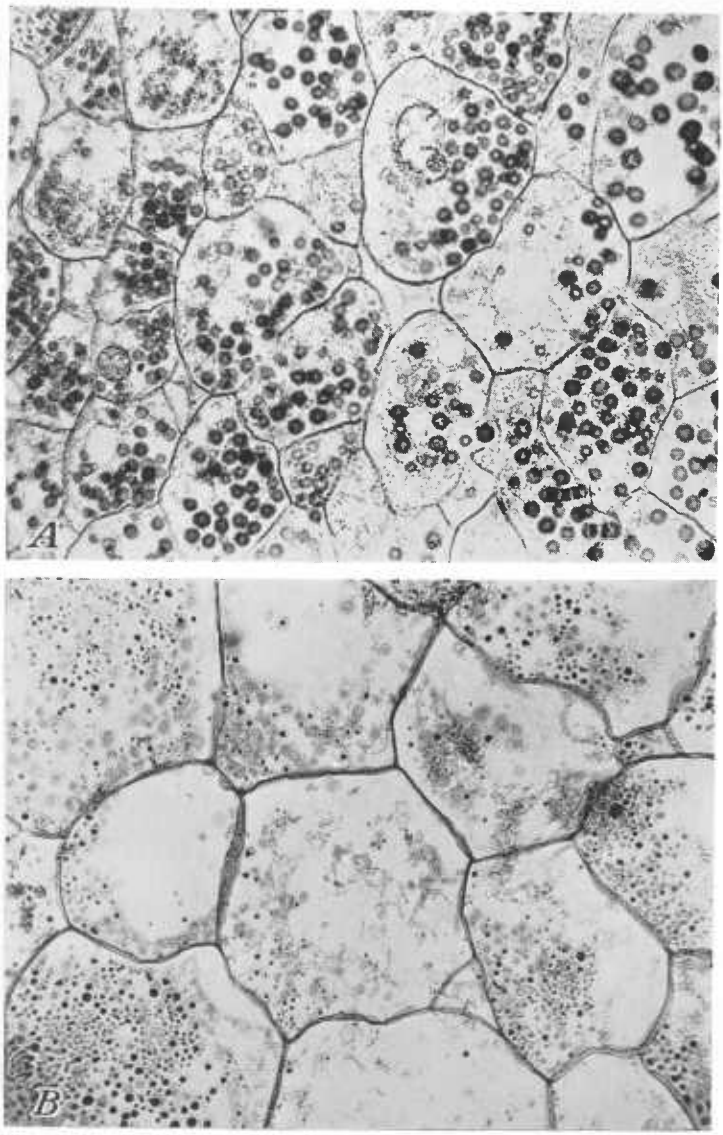
endosperm influenced the development of the character in question, i. e., the carotenoid content of the corn meal. This could be accomplished, since it was readily possible to determine cell volumes, and the degree of development of the character could be accurately determined by quantitative chemical and photometric analyses.

Since there is a twofold increase in the number of genes per cell in the tetraploid as compared with the diploid, the same concentration of the genes per unit volume will be maintained in the tetraploid if the volume of the cells is also doubled. If cell volume is not doubled, the concentration of the genes per unit volume will be increased in the tetraploid; if cell volume is more than doubled, there will be a corresponding decrease in gene concentration.

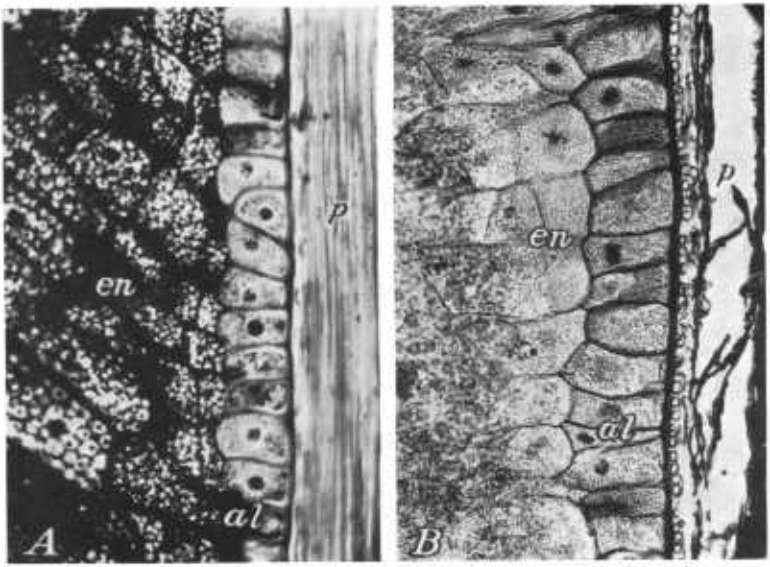
Computations of endosperm cell volume were made from the tissue in the central region of the endosperm and from the peripheral (aleurone) layer of cells in the midabgerminal region of mature kernels that in size and shape were representative of the diploid and tetraploid strains. In the middle region of the endosperm the cells were irregular in outline but isodiametric in longitudinal and cross sections. The relative volume of the cells in this region was computed by treating the cells as spheres whose areas were the areas of the cells in section view, areas being procured from planimeter measurements. The shape of the peripheral cells was essentially rectangular in both longitudinal and cross sections, their width when viewed in cross section of the kernel being somewhat less than their depth when viewed in longitudinal sections of the kernel. The volume of the aleurone cells was computed by multiplying together their three dimensions procured from the cross and longitudinal sections. Photomicrographs of representative regions of the endosperm from which cell measurements were taken are reproduced in plates 1 and 2.

For determining the mean cell volume in the two regions of the endosperm, five groups of six to eight contiguous cells were measured in each of four diploid and four tetraploid kernels selected as representative of the two strains. The values for cell volume obtained by this method of measuring all of the members of a given group of cells were somewhat lower than the true values, since the plane of section ordinarily was not median for all of the cells measured. Thus the maximum dimensions of all of the cells were not procured. The values are relative, not absolute, and provide an adequate basis only for a comparison of volume relations in the two kinds of corn. The computations of cell volume are given in table 2, including the means for each kernel and the means for all of the measurements from the two regions of the diploid and tetraploid kernels, together with their standard errors.

From the data presented in table 2 it is apparent that the endosperm cells in the tetraploid were very much larger than those of the diploid, the ratio of their volumes being essentially 3.6 : 1 in both the peripheral aleurone layer and in the central region. This is a much greater increase than has been reported in other studies of cell-volume relations following chromosome doubling. Nuclear volume in the same cells, as estimated from a limited number of measurements, was in the ratio of approximately 2.5 : 1, indicating that the ratio of chromosome numbers was 2 : 1 as expected in the endosperm of the diploid and tetraploid kernels that were examined.



Photomicrographs of endosperm tissue from the central region of the kernel in (*A*) diploid and (*B*) tetraploid corn taken at the same magnification. × 260.



Photomicrographs of the aleurone layer and adjacent tissues from kernels of (*A*) diploid and (*B*) tetraploid corn taken at the same magnification. Longitudinal sections from the midabgerminal region of the kernel: *al*, Aleurone; *en*, endosperm; p, pericarp. × 260.

TABLE 2.—*Comparisons of cell volume* [1] *in the endosperm of diploid and tetraploid corn*

Kernel No.	Central region		Aleurone layer	
	2*n*	4*n*	2*n*	4*n*
	Cubic millimeters	*Cubic millimeters*	*Cubic millimeters*	*Cubic millimeters*
1	175,478	645,068	5,155	18,380
	163,051	708,896	6,380	20,950
	153,710	549,181	6,730	15,700
	170,673	418,469	5,085	19,660
	163,051	755,536	4,655	17,420
Mean	165,193	615,430	5,601	18,422
2	157,960	448,943	4,245	21,160
	148,036	410,405	6,115	20,670
	145,132	349,585	4,490	22,600
	138,685	413,081	5,360	23,740
	180,374	571,698	5,730	31,280
Mean	154,037	438,742	5,188	23,890
3	119,560	469,084	6,360	18,080
	118,036	397,197	5,970	19,290
	141,118	386,833	7,120	20,820
	126,108	693,150	6,400	21,310
	188,455	575,773	6,365	23,800
Mean	138,655	504,407	6,443	20,660
4	143,285	610,112	4,870	21,240
	127,342	560,238	4,465	18,110
	152,216	490,720	6,020	17,260
	137,910	701,160	5,250	19,060
	128,782	530,272	5,830	20,750
Mean	137,907	578,500	5,287	19,284
Mean of all measurements	148,948 ±4,527	534,269 ±26,850	5,630 ±179	20,563 ±739
Ratio	1 : 3.58		1 : 3.65	

[1] Volumes were computed in cubic millimeters from measurements procured at a magnification of 530 diameters.

Although the individual cells of the tetraploid contained twice as many genes as did the cells of the diploid, the number of genes per unit volume was actually less in the endosperm of the tetraploid than in the diploid, owing to the pronounced increase in the volume of the cells of the tetraploid. Conversely, since the reduction in gene number per unit volume in the tetraploid was associated with a marked increase in carotenoid content per unit volume there was a very significant increase in the amount of carotenoid per gene elaborated by the tetraploid, and a still greater increase in the amount of carotenoid per cell. These proportional differences between the endosperm of the diploid and tetraploid strains are shown in table 3.

TABLE 3.—*Proportional differences between the endosperm of diploid and tetraploid strains of yellow corn*

Item	Diploid	Tetraploid	Item	Diploid	Tetraploid
Cell volume	1	3.6	Genes per unit volume	1	0.55
Carotenoid per unit volume	1	1.4	Carotenoid per cell	1	5
Genes per cell	1	2	Carotenoid per gene	1	2.5

The disproportionate increase in the size of the endosperm cells of the tetraploid, which were 3.7 times as large as those of the diploid, may have been due to their hexaploid constitution with which was associated a retarded nuclear and cell-division activity and a compensating increase in cell enlargment. Evidence in support of this view is furnished by octaploid corn plants, which invariably are much reduced in stature and have cells that are relatively very large and few in number. However, the kernels of the tetraploid were 50 percent larger than those of the diploid, and this increase was roughly proportional for the constituent parts of the kernel, including the endosperm, embryo, and pericarp. It is not known to what extent the increased size of the hexaploid endosperm was conditioned by the influence of other parts of the kernel that were tetraploid in chromosomal constitution.

The fact that in the tetraploid there was more than a twofold increase in the amount of carotenoid per gene could be interpreted to mean that a doubling of the number of genes per cell more than doubles the efficiency of each gene. But it might be argued that the endosperm is primarily a storage tissue and that the amount of carotenoid which it contains is conditioned by relationships existing in other tissues of the plant where carotenoids are being synthesized. For example, the leaf tissue contains appreciable amounts of the same carotenoids that are present in the endosperm and it is conceivable that the endosperm serves merely as a storage organ for a portion of the carotenoids synthesized by the leaves. However, it was shown by Johnson and Miller (*3*) that the amount of carotenoid in the leaf tissue of white- and yellow-endosperm sister lines was essentially the same, but the endosperm of the white lines contained very small amounts of carotenoid. Their work also substantiated the earlier results of Mangelsdorf and Fraps (*8*) to the effect that there is a positive correlation between the number of dominant genes for yellow and the amount of carotenoids in the endosperm of ordinary diploid corn. Thus it appears that the carotenoid content of the endosperm tissue is determined by the number of dominant genes for yellow endosperm present in the tissue rather than by conditions existing elsewhere in the plant; furthermore, from the results of the present investigation it may be concluded that the number of genes per cell unit is of more importance than the number of genes per unit volume in determining the amount of carotenoids present in the endosperm.

DISCUSSION

The results reported here have demonstrated a percentage increase following chromosome doubling in the carotenoids of yellow corn, and a percentage decrease in the carotenoids of white corn. Obviously, when there is a percentage increase in some of the substances in the corn kernel there must be a corresponding percentage decrease in other components, and vice versa. The effect of chromosome doubling on the relative amounts of other important constituents of the corn kernel, such as carbohydrates, proteins, and fat, was not determined in this investigation.

The percentage increase in the carotenoid content of the tetraploid yellow corn is interpreted as being due to a cumulative action of the dominant genes for yellow endosperm color. Since these dominant genes were not present in the white corn in an effective combination to

produce well-developed endosperm color, cumulative action was lacking in the tetraploid white corn and there was a resultant percentage decrease in carotenoids.

These results suggest that there are two categories of gene action in autotetraploids: (1) Cumulative gene action, which yields percentage increases and accounts for the distinctive traits of autotetraploids other than those that may be attributed directly to the presence of an increased number of chromosome sets, and (2) noncumulative gene action, which yields percentage decreases when the percentages of other constituents are increased. The significance of this classification can be understood better in the case of corn carotenoids if the quantity of pigment is expressed in amount per cell rather than in percentage. In the tetraploid yellow corn, owing to cumulative gene action, there was a fivefold increase in the amount of carotenoids per cell. In the tetraploid white corn, if it is assumed that the cell-volume relations in the diploid and tetraploid were the same as in the yellow corn, there was an increase in the amount of carotenoid per cell as a result of chromosome doubling; but this increase was very much less than in the yellow corn, owing to the absence of cumulative gene action. Further study of the differences that distinguish chromosome-doubled strains from their parent strains is needed, based on analyses of individual traits which may or may not be conditioned by cumulative gene action.

The relative importance of autotetraploids as horticultural and crop plants will be determined by the extent to which desirable traits are accentuated or produced by chromosome doubling without accentuating or producing undesirable traits. In terms of gene action, this means that chromosome-doubled strains of cultivated plants may have increased value if their desirable traits are controlled by genes that function in a cumulative manner to yield significant percentage increases like those reported here for the carotenoids of yellow corn. The fact that most of the more important crop plants are polyploids suggests that cumulative gene action has been an important determining factor in the evolution of cultivated plants.

SUMMARY AND CONCLUSIONS

Doubling the number of chromosomes in pure yellow corn caused a 40-percent increase in the content of carotenoid pigment.

The active provitamin A fraction of the carotenoids, including beta-carotene and cryptoxanthin, was increased in the tetraploid yellow corn approximately in proportion to the increase in total carotenoid pigment.

The volume of the endosperm cells of the tetraploid was approximately 3.6 times the volume of the endosperm cells of the diploid.

The increase in the cell volume and the carotenoid content of the endosperm in the tetraploid yellow corn resulted in a fivefold increase in the amount of carotenoid per cell.

The genes for yellow endosperm exerted a cumulative action following chromosome doubling. In the individual endosperm cells of the tetraploid the amount of carotenoid elaborated per gene was 2.5 times as great as in the individual cells of the diploid, even though there was a greater concentration of genes per unit volume in the diploid than in the tetraploid.

Doubling the number of chromosomes in white corn decreased the carotenoid content 19 percent. With respect to carotenoid content there was no cumulative gene action in the white corn.

The carotenoid content varied widely among different commercial varieties, inbred strains, and hybrids of ordinary diploid yellow corn. The inbred line with the highest carotenoid content had more than four times as much carotenoid as the line with the lowest carotenoid content.

The yellow appearance of the kernel was not a reliable criterion of carotenoid content.

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