Proc. Natl. Acad. Sci. USA
Vol. 82, pp. 7010–7014, October 1985
Genetics

DNA amplification patterns in maize endosperm nuclei during kernel development

(chromatin/polyploidy/polytenization/replication/cytophotometry)

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Communicated by John D. Axtell, July 1, 1985

ABSTRACT **Increased DNA levels in centrally located endosperm nuclei are shown to be related to endosperm development in *Zea mays*. Mitotic activity sharply decreases in endosperm cells 10–12 days after pollination. At this time nuclear size and DNA content per nucleus (where C = haploid content) sharply increase until peak levels are reached at about 14–18 days after pollination. Mean DNA content per endosperm nucleus in strain A188 was shown by Feulgen cytophotometry to increase to about 90C by this peak stage, with the pattern being remarkably consistent over four consecutive growing seasons. Some individual nuclei achieved levels of >200C. Most other strains compared during one growing season averaged even higher peak levels of DNA per nucleus than did A188. Individual nuclei in those strains reached levels as high as 690C. A decrease in DNA level was observed in older endosperms with most strains. Endosperm mutant strains did not show a significant reduction in DNA. Opaque-2 mutants in several backgrounds achieved higher levels of DNA per nucleus. DNA levels from F_1 endosperms did not indicate heterosis. Regardless of differences in DNA content, the pattern of DNA increasing as development proceeds followed by a DNA decrease was observed for most strains. Cytological studies reveal much variation in chromatin strandedness, a maximum of three nucleoli, a maximum of three nucleolar organizer regions, and ≈30 diffuse chromatin masses in older endosperm tissue. A form of DNA amplification, perhaps polytenization, appears to be occurring during endosperm development.**

The developmental biology of maize (*Zea mays* L.) is important because of the prominence of maize in plant genetics research and its importance as a major agricultural food crop. Understanding the development of the endosperm of maize becomes especially important because this tissue makes up 85–90% of the mature kernel dry weight. Endosperm tissue obviously serves a critical role in overall kernel development.

The initial ploidy level of the endosperm is 3X, because two polar nuclei of the central cell of the embryo sac fuse with one of the two sperm nuclei of the pollen grain. Within several hours, the initial triploid nucleus undergoes rapid and synchronous divisions that ensue for several days. Both nuclear divisions and cytokineses eventually cease in the more central regions of the tissue, whereas these activities persist for the longest time only in the peripheral endosperm regions. The outermost layer of cells, the aleurone, cytologically behaves like a meristem providing additional cells to the interior region of the kernel. The nuclei of the nonperipheral cells continue to increase in size during the early period of endosperm development. Aside from the morphological aspects of kernel development reported by Lampe (1), Randolph (2), and Kiesselbach (3), the genetic and molecular behaviors of maize endosperm during the process of development have not been fully described. Nuclei of the central endosperm regions have been characterized with regard to nuclear size, chromosome knobs, nucleoli, and chromatin strandedness (4). DNA increases were suggested based primarily upon observed increases in nuclear size. Similar observations have been made for maize endosperm cells grown under *in vitro* conditions (5). Reports based only upon nuclear volume indicated increased DNA contents ranging from 6C to 384C (6, 7). One report based upon Feulgen cytophotometry showed maximum DNA levels of 24C in kernels of young ears (8). None of these investigations quantified DNA levels in relation to endosperm development.

MATERIALS AND METHODS

Stocks and Sample Collections. Inbred A188 plants were grown during the 1980–1983 growing seasons at the University of Minnesota, St. Paul. Other strains were grown during the 1983 season. Kernels were collected at 1- to 3-day intervals following self- or cross-pollinations and were immediately fixed in 95% ethanol/glacial acetic acid, 3:1.

Cytological Observations. Squashes were made with propionic carmine stain. Mean nuclear volume was calculated from measurements made with a Zeiss digital image analyzer system (MOP-3). Mitotic index was calculated as the fraction of the total number of cells in mitosis.

Autoradiography. Three kernels from ears at 8, 12, or 16 days after pollination (ap) were placed in 1.5 ml of sterile modified kernel medium (9). After moderate shaking at 28°C for 2 hr, 20 μl of [^{3}H]thymidine was added at a concentration of 1 mCi/ml (specific activity, 80.1 Ci/mmol; 1 Ci = 37 GBq). Incubation was continued for 16–20 hr on a shaker at 28°C. Kernels were fixed in 95% ethanol/glacial acetic acid, 3:1. Centrally located endosperm tissue was squashed on slides in a drop of 45% acetic acid. Cover glasses were removed by the dry ice method. The preparations were then subjected to a 70%, 50%, and 30% ethanol series and 0.3 M NaCl/30 mM sodium citrate, pH 7.5, for 5 min each. Slides were dipped into Kodak NTB-2 liquid emulsion diluted 2:1 with distilled water at 42°C, dried for 2 hr, placed into an opaque slide box with drierite, and exposed at 4°C for 7 days. Development was accomplished with D-19 developer for 1 min, stop bath for 30 sec, Kodak fixer for 2 min, and washing for 5 min. The slides were placed into 5% Giemsa for 6 min, briefly rinsed in 0.01 M phosphate buffer (pH 6.9), air-dried, and mounted in Euparal.

Cytophotometry. Slides were prepared with endosperm tissue fixed in 95% ethanol/glacial acetic acid, 3:1. Tissue was taken from only the central endosperm regions for those

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Abbreviation: ap, after pollination.

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samples large enough to facilitate such tissue removal—that is, 10 days ap and later. This tissue was placed in a drop of 45% acetic acid and squashed on slides that also contained fixed *Gallus* (chicken) erythrocytes as an internal reference (10). Cover glasses were removed by the dry ice method, which also removed much of the starch from around the nuclei. Slides were immediately subjected to the Feulgen reaction series and made permanent according to the following schedule: 5 min each in 70%, 50%, and 30% ethanol and distilled water; hydrolysis in 5 M HCl for 20 min at room temperature; cold distilled water for 5 min; Schiff's reagent for 1 hr; bleach (distilled water/1 M HCl/10% potassium metabisulfite, 18:1:1) for 10 min; and 5 min each in distilled water, 30%, 50%, 70%, 95%, and 100% ethanol. Slides were then mounted in Euparal.

A two-wavelength microspectrophotometric method was employed (11, 12) with a Zeiss Universal microscope with an MP 101 photometer. From spectral curves, 550 nm and 488 nm were selected as the two appropriate wavelengths. Percent transmission at each of the two wavelengths was obtained for both endosperm nuclei and chicken erythrocyte nuclei on the same slide and then was converted into arbitrary DNA units (13). A mean DNA content per chicken erythrocyte of 5.33 pg was determined with the use of an interference microscope at the U.S. Department of Agriculture Forest Service, North Central Forest Experiment Station (Rhinelander, Wisconsin). This value contrasts somewhat with other reports that vary between 2.5 and 3.97 pg (10; 14–16). Calculations of DNA (pg) per endosperm nucleus were based upon arbitrary DNA units measured for endosperm nuclei and chicken erythrocyte nuclei and the known amount of DNA per chicken erythrocyte.

These values were converted to DNA C levels (where C = haploid content) by dividing the pg of DNA per maize nucleus by 5.035 pg, the amount of DNA determined to be in a haploid maize nucleus (17). This DNA value was confirmed by measuring root-tip anaphase figures (2N) compared with the chicken erythrocytes.

RESULTS AND DISCUSSION

Rapid development of endosperm tissue occurs in the kernel following pollination (Fig. 1*A*). Particular note can be made of the changes in overall endosperm size that occur between 8 and 12 days ap; this is due to an increase in cell number and, to some extent, cell and nuclear size. Thereafter, increases in cell and nuclear size rather than cell number are the basis for changes in the central endosperm region. Microscopic studies of centrally located endosperm tissue reveal extremely large nuclei, especially later than 12 days ap. An indication of the extent to which these nuclei can develop is given in Fig. 1*B*.

The mitotic index for the central endosperm tissue peaked 8–10 days ap and then sharply decreased. Only 1 of 413 nuclei located in the central region was found to be undergoing mitosis 14 days ap or later. Nonmitotic endosperm nuclei of these later developmental stages were shown by [^{3}H]thymidine autoradiography to be actively synthesizing DNA. Silver grains were observed directly over nuclear chromatin at stages later than 12 days ap. Some of the [^{3}H]thymidine incorporation could be the result of DNA repair.

In A188, the strain most intensively studied, DNA level per nucleus and nuclear size surge 8–10 days ap and peak between 14 and 18 days ap. DNA content per nucleus reached a maximum mean level of 90–100C. Nuclear volume and DNA content means for each ap date are significantly correlated ($r = 0.92$; $P < 0.001$). DNA content of interphase nuclei measured before the rapid increase averaged between 4 and 5.6C. Some of these nuclei were presumably in the S or G_2 phase of the nuclear cycle, at least partially accounting

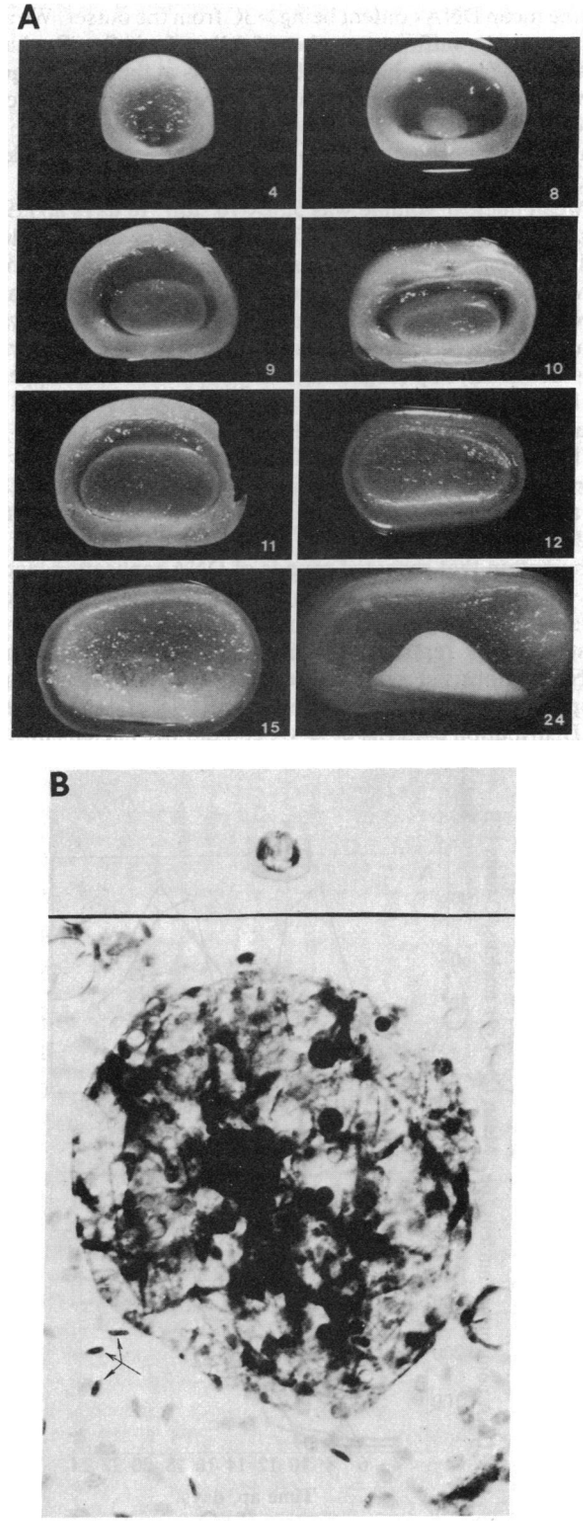

FIG. 1. (*A*) Development of the endosperm as illustrated by kernel cross sections. Four days ap, the endosperm is the small structure at the kernel base. The bulk of the kernel consists of nucellar tissue with surrounding carpels; 8 days ap, the endosperm is easily discerned as the body in the lower part of the kernel; 9–12 days ap, the endosperm has compressed the nucellus toward the outer edge of the kernel; 15 days ap, the volume of the kernel is mostly endosperm; 24 days ap, the embryo is additionally observed within the endosperm mass. (*B*) A maize root-tip nucleus (top) compared with a 22-day ap endosperm nucleus (bottom) against a background of chicken erythrocyte nuclei (arrows). (Nuclear stain, propionic carmine; original magnification, ×200.)

for the mean DNA content being >3C from the outset. We are not sure that a sufficient number of cells were in S or G_2 phase to account for the relatively high C levels at the early stages. A possibility is that some cells may have already commenced DNA replication in the absence of cytokinesis. Fig. 2*A* illustrates the timing relationships among mitotic index, nuclear volume, and DNA per nucleus for inbred A188.

Mean nuclear volume was 5.3×10^4 μm^3 18 days ap (1981 data); this constitutes a 20-fold increase in mean nuclear volume compared to 4 days ap. Regardless of different environmental conditions from year to year, the pattern of DNA increases per nucleus over four consecutive growing seasons showed remarkable similarity (Fig. 2*B*). The period of ≈10–12 days ap (beginning 8 days ap in one case) appears to be a critical time in endosperm development marked by drastic changes in DNA levels.

The rate of DNA amplification during the 10- to 16-day ap period of endosperm development is noteworthy. In the A188 strain, the mean increase in DNA per nucleus during this 6-day period was about 480 pg (1981 data). Based on the assumption that repeated rounds of DNA replication lead to an exponential increase, each round of replication is accomplished in ≈22 hr over this time period. This means that the last round of replication during this period requires linear DNA synthesis at a rate of 916.4 μm/sec per nucleus, which approximates 2.75×10^6 base pairs per second per nucleus.

Distribution patterns of DNA content per nucleus in A188

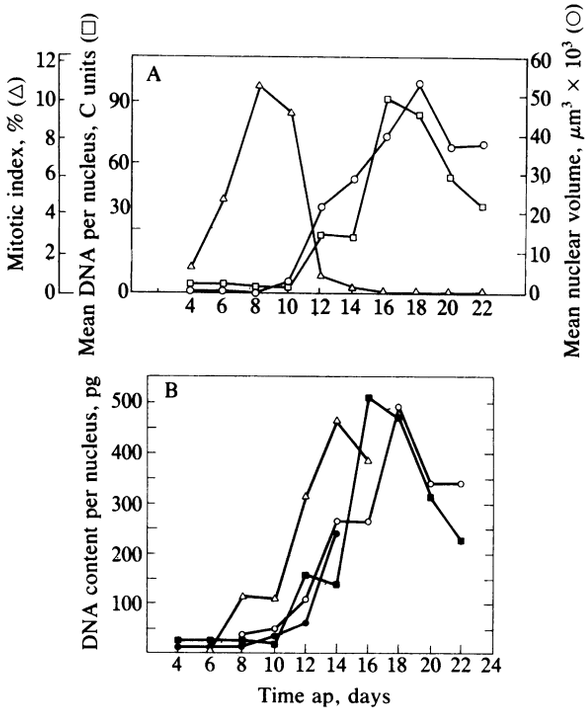

FIG. 2. (*A*) Mitotic index (△), DNA content (□), and nuclear volume (○) for 4-day through 22-day ap maize (A188) endosperm. Mitotic index is based on 352–970 cells per ap date. DNA content and nuclear volume are based on 20–47 nuclei per ap date. (*B*) DNA content per nucleus from centrally located A188 endosperm tissue over four consecutive growing seasons: 1980 (●), 1981 (■), 1982 (○), 1983 (△). The 1980 measurements were only made through 14 days ap, and the DNA (pg) in that year was based upon reference nuclei (*Gallus* erythrocytes) prepared on separate slides. All other calculations were made by using *Gallus* erythrocytes prepared on the same slide (internal reference). An earlier increase and peak in DNA content occurred in 1983 than in the other three growing seasons, all of which began a rapid DNA increase at about 10 days ap.

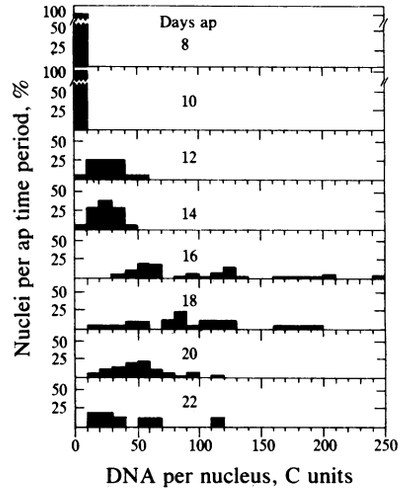

FIG. 3. Distribution patterns of DNA content of centrally located A188 nuclei over the 8-day to 22-day ap period. Note that some of the nuclei contain >200C amounts of DNA at 16 days ap.

for the ap periods are displayed in Fig. 3. Distribution patterns of nuclear size are very similar to those of DNA content. Some nuclei at 16 and 18 days ap reach DNA levels of >200C. The distributions also show that such large nuclei are not found at the earlier dates ap, all of which minimizes the possibility that the DNA content pattern could be due to erroneous sampling.

Such DNA increases raise the question of whether the larger nuclei result from nuclear fusion, polyploidy (>30 chromosomes), polytenization (endoreduplication), underreplication, or preferential gene amplification. From squashes and sections of endosperm tissue, Duncan and Ross (4)

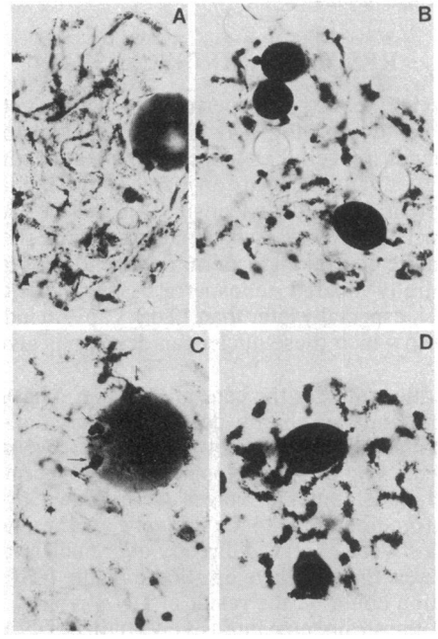

FIG. 4. Cytological aspects of endosperm nuclei. (*A*) An A188 endosperm nucleus at 22 days ap depicting various degrees of chromatin strandedness. (*B*) Three prominent nucleoli in an endosperm nucleus. (*C*) One large nucleolus showing three distinct nucleolar organizer regions (arrows). (*D*) An 18-day ap endosperm nucleus from A188 with ≈30 diffuse chromosome-like bodies. (Nuclear stain, propionic carmine; original magnifications: *A–C*, ×500; *D*, ×200.)

observed chromatin multistrandedness, never more than three nucleoli, a constant knob number, and ≈30 chromosome-like bodies in older tissue. They concluded that the endosperm cells were basically triploid and that the vast increases in nuclear size were due to endomitotic events. Tschermak-Woess and Enzenberg-Kunz (7) reported occasional smaller nucleoli, called secondary nucleoli, alongside of the main large nucleolus. Still, their photomicrographs show only three deeply stained nucleolar organizer regions per nucleus.

Our observations also show chromatin multistrandedness in nuclei of 12-day ap and older endosperm (Fig. 4*A*). Much variation exists in chromatin strandedness—that is, from areas of single strands to areas of many strands with chromomeres sometimes appearing in a side-by-side orientation. Individual chromosomes cannot be followed throughout their length after mitotic activity ceases at about 14 days ap because of the lack of condensation. A variety of chemical pretreatments over temperature gradients did not successfully individualize the chromosomes. In addition, we always observed one, two, or a maximum of three fully developed nucleoli per endosperm nucleus (Fig. 4*B*). Three nucleolar organizer regions were always observed in nuclei with only one large nucleolus (Fig. 4*C*). In older tissue, some nuclei were observed with ≈30 diffuse chromosome entities consistent with a 3X chromosome number in maize (Fig. 4*D*). These chromosome-like structures, or chromatin masses, are much wider than normal maize chromosomes. *In situ* hybridization of maize endosperm nuclei with labeled rRNA resulted in only three silver grain clusters over nucleoli (18).

The cytological investigations indicate that polyploidy (beyond triploidy) is unlikely as the main condition of these particular endosperm cells. Punnett (6) reported some hexaploid cells in 8-day ap tissue from greenhouse-grown plants. No hexaploid cells were reported in the 10- to 14-day ap time period. The excellent cytological techniques of Lin (19, 20) revealed 95% of the nuclei to be triploid in 6- to 10-day ap tissue. The other 5% consisted mostly of 6X, 9X, and an infrequent number of 12X. Lin provided evidence that endosperm tissue is not uniformly triploid and proposed nuclear fusion as a possible explanation. We have also observed occasional nuclei that appeared to be polyploid. Although a polyploid condition beyond triploidy may exist in a small number of nuclei, especially in younger tissue and in the more peripheral regions, the overall cytological evidence has led us to discount conventional polyploidy as the main explanation for the high DNA levels in the central regions of the endosperm. We suggest that, at the minimum, some form of polytenization occurs in maize endosperm nuclei during development. Polytene chromosomes have been found in suspensor cells, cotyledons, antipodals, synergids, and other tissues in a number of plant species (21, 22).

Mean DNA content per nucleus during endosperm development is presented in Table 1 for 17 different strains, including endosperm mutants in various backgrounds and F_1 crosses. In all strains, the mean DNA content per nucleus

Table 1. Mean DNA per nucleus (C levels) during endosperm development for 17 maize strains

Time ap, days	Number of nuclei	C level
	A188	
6	20	6 ± 0.3
8	41	22 ± 2.0
10	42	22 ± 2.3
12	41	62 ± 5.1
14	30	93 ± 8.7
16	29	68 ± 7.0
18	43	67 ± 8.9
20	17	49 ± 7.9
	B37	
8	33	23 ± 2.9
12	47	37 ± 4.8
14	45	66 ± 7.8
16	45	95 ± 8.0
19	43	141 ± 17.5
	L289	
9	18	30 ± 5.8
13	45	94 ± 8.9
15	45	155 ± 16.7
17	45	80 ± 6.9
	Wilbur's Knobless Flint	
8	44	12 ± 1.0
12	32	78 ± 10.8
15	52	137 ± 12.6
17	46	147 ± 11.4
19	42	125 ± 11.0
	Zapalote Chico	
8	40	18 ± 2.5
12	40	65 ± 8.9
14	50	47 ± 4.8
16	37	55 ± 4.8
19	42	112 ± 10.2
	A188 opaque-2	
9	32	8 ± 0.9
13	25	114 ± 23.6
	A188 waxy	
7	22	8 ± 1.3
9	21	3 ± 0.2
11	23	12 ± 1.4
13	23	55 ± 10.9
	B37 waxy	
11	21	54 ± 6.5
13	33	22 ± 2.1
16	42	59 ± 5.5
17	40	123 ± 13.3
19	48	134 ± 13.9
	B37 floury-2	
9	32	13 ± 1.2
11	33	19 ± 2.3
13	41	93 ± 12.3
15	20	106 ± 25.5
17	40	161 ± 13.6
19	39	162 ± 16.1
	B37 opaque-2	
9	15	40 ± 7.6
11	32	43 ± 4.3
13	35	57 ± 6.2
15	34	72 ± 10.2
17	45	178 ± 19.1
19	44	168 ± 15.0
20	45	114 ± 10.6
	B37 opaque-2 floury-2	
8	24	6 ± 1.0
10	43	30 ± 2.8
12	69	26 ± 1.6
14	42	58 ± 5.0
	B37 sugary-2	
9	23	15 ± 2.0
11	25	45 ± 6.8
19	28	80 ± 9.1
	A619 opaque-2	
9	22	6 ± 0.4
11	26	42 ± 5.7
13	27	33 ± 3.0
18	22	153 ± 24.6
20	30	103 ± 14.4
	A188♀ × B37♂ (F_1)	
8	33	32 ± 4.3
10	41	57 ± 7.0
14	22	35 ± 8.5
15	35	55 ± 6.7
	B37♀ × A188♂ (F_1)	
8	23	21 ± 2.4
10	23	23 ± 3.4
12	41	67 ± 8.0
14	31	57 ± 9.0
16	43	78 ± 8.9
17	42	57 ± 5.4
	Zapalote♀ × Wilbur's Flint♂ (F_1)	
9	41	30 ± 2.7
12	21	66 ± 8.5
15	23	120 ± 18.7
18	32	87 ± 7.6
	Wilbur's Flint♀ × Zapalote♂ (F_1)	
10	42	34 ± 3.0
12	24	57 ± 7.9

C levels are expressed as means ± SEM. All strains and crosses have highly significant differences in DNA levels among days ap.

varies over the different ap periods in a highly significant manner. These highly significant deviations exist in spite of the tremendous variation in DNA content among the nuclei within each ap date (see Fig. 3). The mean DNA content per nucleus is drastically increased in the central tissue of the endosperm during development.

Opaque-2 in a B37 background possessed the highest peak for mean DNA content per nucleus, reaching 178C. This is almost twice as much DNA per nucleus as that attained by A188, the line initially investigated. A188 may prove to be one of the lower DNA strains. Opaque-2 in the A619 background also showed a relatively large amount of DNA per nucleus, peaking at 153C. The floury-2 mutant in the B37 background reached a value of 162C. Both opaque-2 and floury-2 in B37 reached higher mean DNA contents per nucleus than the B37 inbred without an endosperm mutation, although B37 may not have reached the peak at the dates sampled. The highest value for an individual nucleus was 690C observed in opaque-2 with an A188 background; opaque-2 in the B37 background had a nucleus with a 635C DNA level. With the possible exception of B37 sugary-2, endosperm mutations do not appear to dramatically alter the mean DNA per endosperm nucleus over the course of development. The B37 sugary-2 strain must be investigated further, especially since sugary-2 reduces seed size (23).

Four seasons of data for A188 indicate that the mean DNA level decreases somewhat after achieving the peak at about 14–18 days of endosperm development. Several of the strains reported here also appear to decrease in mean DNA levels at later stages of endosperm development—namely, L289, Wilbur's Knobless Flint, Zapalote × Wilbur's Flint, B37 × A188, B37 opaque-2, and A619 opaque-2. This decrease could be due to DNA degradation rather than preferential loss of larger nuclei or sampling error. The other strains may not have been sampled late enough to have reached the peak. The overall patterns, however, are consistent in demonstrating an initial low DNA level and increasing levels during development at least to a point around 14–18 days ap.

Values of maximum mean DNA content per nucleus were statistically compared for various groups of interest. An F test of all of the strains indicated highly significant differences in maximum DNA values. The five strains A188, B37, L289, Wilbur's Knobless Flint, and Zapalote Chico reveal a rather large range and highly significant differences in maximum DNA levels. B37 and A619 converted to opaque-2 achieve high mean DNA levels of 178C and 152C, respectively. The A188 opaque-2 strain was only sampled through 13 days ap. Nonetheless, the 114C for opaque-2 in A188 at 13 days ap surpassed all maximum mean DNA levels observed in the A188 strain without an endosperm mutation over four consecutive growing seasons. The endosperm DNA level resulting from the F_1 cross of Zapalote Chico and Wilbur's Knobless Flint was very similar to the lower value of the two parents—namely, Zapalote Chico (Table 1). Other F_1 crosses were made between the B37 and A188 strains. From the reciprocal crosses, the resultant endosperm possessed lower DNA levels per nucleus than the parental strains. In these few preliminary F_1 crosses, at least, heterosis does not appear to exist relative to DNA content per endosperm nucleus.

Foremost among the questions prompted by these results is that relating to the purpose of the extensive DNA amplification. Certain genes such as the rRNA genes, for which multiplicity is deemed necessary for highly active synthesis, are already found to number between 5000 and 12,000 per 2C nucleus (24) without additional amplification.

The maize endosperm is extremely heterogeneous within a single kernel relative to cell size, nuclear DNA content, and, to some extent, morphological appearance. How this heterogeneity relates to gene expression during endosperm development is another question. The repeatable nature of the DNA increase indicates that it is fundamental to kernel development. Also, since kernel development is fundamental to yield *per se*, understanding the plasticity of the maize nuclear genome during development may lead to useful applications in genetics and breeding research.

This is paper 13,407, Scientific Journal Series, Minnesota Agricultural Experiment Station (St. Paul, MN). Additional support was provided by grants from the National Science Foundation (PCM-82155757), Pioneer HiBred International, Inc. (Des Moines, IA), and the McKnight Foundation.

1. Lampe, L. (1931) *Bot. Gaz. (Chicago)* **151,** 337–377.
2. Randolph, L. F. (1936) *J. Agric. Res. (Lahore)* **53,** 881–916.
3. Kiesselbach, T. A. (1949) *Nebr. Agric. Exp. Stn. Res. Bull.* **161,** 1–96.
4. Duncan, R. E. & Ross, J. G. (1950) *J. Hered.* **41,** 259–268.
5. Straus, J. (1954) *Am. J. Bot.* **41,** 833–839.
6. Punnett, H. H. (1953) *J. Hered.* **44,** 257–259.
7. Tschermak-Woess, E. & Enzenberg-Kunz, U. (1965) *Planta* **64,** 149–169.
8. Swift, H. (1950) *Genetics* **36,** 643–654.
9. Gengenbach, B. G. (1977) *Planta* **134,** 91–93.
10. Dhillon, S. S., Berlyn, G. P. & Miksche, J. P. (1977) *Am. J. Bot.* **64,** 117–121.
11. Mendelsohn, M. L. (1958) *J. Biophys. Biochem. Cytol.* **4,** 407–414.
12. Berlyn, G. P. & Cecich, R. A. (1976) in *Modern Methods in Forest Genetics*, ed. Miksche, J. P. (Iowa State University Press, Ames, IA), pp. 1–18.
13. Mendelsohn, M. L. (1958) *J. Biophys. Biochem. Cytol.* **4,** 415–424.
14. LeGal, M. F., Hallet, J. N. & Rey, L. (1984) *Protoplasma* **120,** 113–122.
15. Dhillon, S. S. & Miksche, J. P. (1983) *Histochem. J.* **15,** 21–37.
16. Resslar, P. M., Stucky, J. M. & Miksche, J. P. (1981) *Am. J. Bot.* **68,** 149–153.
17. Phillips, R. L., Weber, D. F., Kleese, R. A. & Wang, S. S. (1974) *Genetics* **77,** 285–297.
18. Phillips, R. L., Wang, A. S. & Kowles, R. V. (1983) *Stadler Genet. Symp.* **15,** 105–118.
19. Lin, B.-Y. (1975) Dissertation (University of Wisconsin, Madison).
20. Lin, B.-Y. (1977) *J. Hered.* **68,** 143–149.
21. Nagl, W. (1981) *Int. Rev. Cytol.* **73,** 21–53.
22. Nagl, W. (1982) in *Cell Growth*, ed. Nicolini, C. (Plenum, New York), pp. 619–651.
23. Glover, D. V., Crane, P. L., Misra, P. S. & Mertz, E. T. (1975) in *High Quality Protein Maize*, ed. Bauman, L. F., Mertz, E. T., Carballo, A. & Sprague, E. W. (Dowden, Hutchinson & Ross, Stroudsburg, PA), pp. 228–240.
24. Phillips, R. L. (1978) in *Maize Breeding and Genetics*, ed. Walden, D. B. (Wiley-Interscience, New York), pp. 711–741.