Amer. J. Bot. 72(10): 1610–1617. 1985.

C-BAND HETEROCHROMATIN AND DNA CONTENT IN ZEA MAYS[1]

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ABSTRACT

The number of mitotic chromosomal C-bands, the percent of the genome comprised of C-band heterochromatin, and genome size (4C DNA content) were determined for 22 North American inbred and open-pollinated lines of *Zea mays*. The number of C-bands ranged from 0 in Tama Knobless Flint to 18 in Zapolate Grande. The percent C-band heterochromatin ranged from 0% in Tama Knobless Flint to 16.9% in Tx601. Genome size varied over 23%: Gaspe Flint had the lowest DNA content (9.82 pg), and Zapolate Grande had the highest (12.12 pg). Genome size and the amount of heterochromatin were significantly correlated.

The corn lines were assigned to five maturity zones encompassing a south-to-north range from Mexico to Canada. Significant negative correlations were detected between the amount of C-band heterochromatin and maturity zones, and between DNA content and maturity zones among the lines.

It is speculated that the simultaneous selection by man for earlier maturation and plant size may be related to the lower DNA content of corn varieties adapted to higher latitudes. Such selection for larger plants may have been achieved through selection for more cells, which could result from the shorter mitotic cycle time that correlates with reduced DNA amount.

CORN heterochromatin has been extensively studied since McClintock (1929) reported the existence of large heterochromatic knobs on pachytene chromosomes. Many studies have focused on characterizing various maize lines in terms of their meiotic pachytene knob composition (Longley, 1938; Brown, 1949; Bianchi and Vetturini, 1969; Kato-Y, 1976). Knobs occur at over 20 positions in pachytene chromosomes (Longley, 1938). Knob number varies, both among plants and among populations, and has been negatively correlated with increasing latitude. Brown (1949) reported that northern flints had few, if any, knobs, standard inbreds and American dents had intermediate knob numbers, and southern dents and Mexican strains had high knob numbers. Also, a negative correlation between knob number and altitude has been observed for Central American and Mexican maize (Wellhausen et al., 1952; Bennett, 1976a; McClintock, Kato-Y. and Blumenschein, 1981).

The use of mitotic chromosome C-banding techniques has permitted the relative amounts and position of heterochromatin to be readily calculated for corn chromosomes (Hadlaczky and Kalman, 1975; Ward, 1980; Mastenbroek and de Wet, 1983; Rayburn, 1984). These studies have shown a striking similarity between heterochromatin patterns observed by pachytene studies and those resulting from C-banding of mitotic chromosomes. The pachytene knobs, for the most part, are apparently the same heterochromatin detected by C-banding.

Peacock et al. (1981) reported that a highly repeated 185 base pair satellite DNA sequence resided in maize knob heterochromatin including abnormal K10. All but six knobs were included in their study. The repeated sequence was not detected in centromeric or NOR heterochromatin, nor in the large blocks of heterochromatin of a B chromosome. The results of Peacock et al. (1981) suggested to us that high knob number strains should have higher DNA amounts, possibly of a magnitude detectable by Feulgen cytophotometric methods.

Bennett (1976a) proposed the following questions: 1) Does significant intraspecific variation in 4C DNA content exist in maize? 2) Does variation in knob number involve variation in 4C DNA amount? 3) Is knob number positively correlated with variation in DNA amount? We report here that both knob number and the amount of heterochromatin positively correlate with 4C DNA content among

[1] Received for publication 8 January 1985; revision accepted 16 May 1985.

Research supported by the Texas A&M Agricultural Experiment Station (Projects 6187, 6371, 6412, and 6703), the National Science Foundation (DEB8009427, H.J.P.; DEB8022173, J.R.G.), and the U. S. Department of Agriculture (GRCO-79-59-2485-1-1-370-1, J.D.S.).

We thank Dr. John Doebley for constructive comments concerning this manuscript.

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maize lines and that a statistically significant, negative correlation exists between DNA content and latitude among 21 lines representing Mexican open-pollinated races, American inbreds, and open-pollinated northern flints.

MATERIALS AND METHODS—*Plant material*—Eighteen inbred lines were examined. Most were well-established lines whose origins were the result of selection out of open-pollinated varieties of corn growing in specific locales; two lines, Tx5855 and Va35, were of more complex pedigree (Table 1; Henderson, 1976). Va35 was chosen as the internal control for genome size determinations because it is a widely used and well-known inbred line. These lines were maintained by either self- or single-sib matings. Additional open-pollinated lines, included because of their known geographical origins, were also examined and are listed in Table 1. The corn lines were assigned "zone" numbers according to their geographical location. These zones were based on maps that divide North America into relative maturity bands (Anonymous, 1978). The five zones used for this study (Fig. 1) were: Zone 1, Mexico; Zone 2, the southern United States; Zone 3, mid-America; Zone 4, the northern United States; and Zone 5, Canada. Zone 2 therefore represents slow-maturing, southern inbred lines, while Zone 4 represents faster-maturing, northern lines. The maturation times are determined in areas where each of these lines is normally grown.

Chromosomal heterochromatin determinations—Seeds of the various lines were germinated in a 1:1 mixture of perlite and vermiculite and incubated in a growth chamber set for a day/night cycle of 8 hr at 30 C and 16 hr at 26 C. After secondary root tips appeared, the seedlings were placed in 10–20 volumes of either 0.05% 8-hydroxyquinoline for 2 hr in the dark at 30 C, or in saturated 5-bromonaphthalene for 24 hr in the dark at 4 C. The seedlings (with the root tips attached) were fixed in 3:1 ethanol:glacial acetic acid for 24 hr at 4 C and stored in 70% ethanol at 4 C.

To prepare chromosomes, fixed root tips were removed from the seedlings, soaked in distilled water for 2–3 min, and hydrolyzed in 0.2 N HCl at 37 C for 10 min. They were rinsed in cold water, placed in a depression well in an agglutinization microslide, and treated with 4–5 drops of an enzyme solution (0.2 g cellulysin, 0.1 g macerase in 10 ml of 1 mM EDTA, pH 5.1–5.6) for 30 min. The root tips were placed on slides following procedures of Rayburn and Gold (1982).

Chromosomes were C-banded using methods similar to those used in corn and other organisms (Filion and Blakey, 1979; Ward, 1980; Mastenbroek and de Wet, 1983). Slides were placed in 5% (saturated) barium hydroxide at room temperature for 3 min, rinsed in running distilled water for 2 min, and placed in humidity chambers in 2 × SSC (0.3 M NaCl + 0.03 M sodium citrate, pH 7.0) at 65 C for 45 min. The slides were rinsed in cold 2 × SSC and in distilled water, stained for 5 min in 2% Giemsa in 10 mM phosphate buffer (pH 6.8), rinsed in distilled water, and air-dried. Chromosomes were observed and photographs were taken through a Zeiss Universal research microscope. Positive prints were made of all C-banded lines. The percentage of heterochromatin (% Het) was determined by measuring the total chromosomal length of the karyotype and dividing it into the total length of the karyotype contained in the C-positive staining areas. The chromosomal measurements were made with the use of a TRS-80 color computer equipped with a digitizer. The program was written by Dr. J. R. Ellison.

DNA content determinations—Seeds were germinated as described above. After 2–3 wk, the fully developed first leaf was removed from the plant and the lower epidermis peeled and fixed in ice cold 3:1 ethanol:glacial acetic acid for 24 hr. After fixation, the epidermis was transferred to 70% alcohol and stored at 4 C. Approximately 5–10 mm squares were placed in a drop of water on a subbed slide (0.2 g gelatin, 0.1 g chrome alum in 100 ml distilled water). A coverslip was placed on the tissue, slight pressure applied, and the coverslip floated off. Epidermis from an internal control (inbred line Va35) was mounted next to the experimental epidermis on the same slide. The slides were hydrolyzed for 40 min in 5 N HCl at 25 C (as determined from the hydrolysis curve), rinsed in distilled water, and stained in Schiff's reagent (prepared exactly as in Price et al., 1980) for 2 hr. After staining, slides were rinsed in two changes of SO_2 water (600 ml distilled water, 36 ml of 1% $K_2S_2O_5$, 30 ml 1 N HCl) for 10 min each and in distilled water for 10 min. After the surplus water was removed, a drop of enzyme solution (0.2 g cellulysin, 0.05 g macerase in 10 ml 1 mM EDTA, pH 5.6) was placed on each epidermis for 30 min. The slides were rinsed in 45% glacial acetic acid, distilled water, and air-dried. After drying, slides were cleared in xylene for 10 min and mounted in Permount with a No. 1 coverslip.

Nuclei were scanned on a Zeiss Universal-

TABLE 1. *Corn lines surveyed in this study, and their origins*

Line	State of origin (zone)[a]	Source	Pedigree
INBRED LINES			
Ar206	AR (2)	Dr. J. O. York Univ. of Arkansas Fayetteville, AR	Tx601-Tuxpan
F6	FL (2)	Dr. E. S. Horner Univ. of Florida Gainesville, FL	Hasting White Prolific × Florida Flint
Ge281	GA (2)	Dr. M. D. Jellum Georgia Exp. Sta. Experiment, GA	Whatley
KYS	KS (3)	Dr. M. P. Maguire Univ. of Texas Austin, TX	Pride of Salina
Ky21	KY (3)	Dr. C. G. Poneleit Univ. of Kentucky Lexington, KY	Boone Co. White
Ky27	KY (3)	Dr. C. G. Poneleit Univ. of Kentucky Lexington, KY	Boone Co. White
Mo8w	MO (3)	Dr. M. S. Zuber Univ. of Missouri Columbia, MO	Cobpipe, open-pollinated
Mo15w	MO (3)	Dr. M. S. Zuber Univ. of Missouri Columbia, MO	Open-pollinated pipe corn
Mp488	MS (2)	Dr. G. E. Scott Miss. State Univ. Miss. State, MS	Yellow Jellicorse
Ms116	MI (4)	Dr. E. C. Rossman Mich. State Univ. E. Lansing, MI	Duncan, open-pollinated
NY16	NY (4)	Dr. G. O. Grogan Cornell Univ. Ithaca, NY	Weber Dent
NY302	NY (4)	Dr. G. O. Grogan Cornell Univ. Ithaca, NY	Bloody Butcher
Pa88	PA (4)	Dr. M. W. Johnson Penn. St. Univ. Univ. Park, PA	Lancaster Surecrop
SD9	SD (4)	Dr. D. B. Shank South Dak. St. Univ. Brookings, SD	Open-pollinated, unknown ear
T8	TN (2)	Dr. L. M. Josephson Univ. of Tennessee Knoxville, TN	Jarvis Prolific
Tx601	TX (2)	Dr. J. D. Smith Texas A&M Univ. Coll. Sta., TX	Yellow Tuxpan
Tx5855	TX (2)	Dr. J. D. Smith Texas A&M Univ. Coll. Sta., TX	(Tx61m × L10)Tx501
Va35	VA (2)	Dr. J. D. Smith Texas A&M Univ. Coll. Sta., TX	(C105 × T8) T8
OPEN-POLLINATED LINES			
Gaspe Flint (GF)	Nova Scotia (5) Peninsular Canada	Dr. R. J. Lambert Maize Genetic Stock Ctr. University of Illinois Champaign, IL	Unknown

TABLE 1. *Continued*

Line	State of origin (zone)[a]	Source	Pedigree
Tama Knobless Flint (TKF)	WI (4)	Dr. R. J. Lambert Maize Genetic Stock Ctr. University of Illinois Champaign, IL	Sac & Fox Indian variety
Nal-Tel (NT)	Mexico (1)	Dr. M. Gutierrez G. CIMMYT Mexico, D.F., Mexico	Ancient endogenous race
Zapalote Grande (ZG)	Mexico (1)	Dr. M. Gutierrez G. CIMMYT Mexico, D.F., Mexico	Prehistoric mestizo race

[a] See Fig. 1.

II scanning microscope with an 03 photometer system, 0.5 μm scanning stage, modified 45-control unit, modified PMI indicator, and a value-averaging module. All measurements were performed at a scanning speed of 8/64 with a damping setting of 5, at 560 nm. DNA values of nuclei of each slide are expressed relative to the mean of five measured nuclei of the internal standard adjusted to 100 Feulgen Absorbancy Units (FAU). Measurements included 15 experimental nuclei per slide, two slides per plant, and two plants per line. A nested analysis of variance was used to evaluate differences between slides, plants, and among lines. Since significant heterogeneity was observed among lines, a Duncan's multiple range test was run to determine the lines that differed. To determine whether significant correlations exist among the various combinations of DNA content, % Het, and geographic zone, Pearson's correlation coefficients were calculated for the various two-way combinations and significance was tested using Student's *t*-test. All statistical analyses were performed using SAS programs run on the TAMU mainframe Amdahl 470V16 computer.

RESULTS—*Chromosomal heterochromatin analyses*—C-banded mitotic metaphase chromosomes were obtained and photographed from 21 lines and karyograms constructed from positive prints. An example of a C-banded karyotype is shown in Fig. 2. C-band number and % Het estimated for the lines are shown in Table 2. K21 was not examined. Five plants of each line were observed for band number. Several lines were observed to contain heterozygous plants (Table 2). Seven of the 17 inbred lines contained heterozygous plants. All five plants of three lines (T8, Ms116, Ky27) were heterozygous for at least 1 C-band. The quantitative heterochromatic composition of the lines examined ranged from 0 C-bands and 0% Het in Tama Knobless Flint to 18 bands and 16.2% Het in Zapalote Grande. Among the inbred lines, the variation ranged from 4 bands and 1% Het in NY302 to 15 bands and 16.9% Het in Tx601. A complete analysis of mitotic metaphase C-band number and position in relationship to pachytene knobs and a fluorochrome analysis of chromosomes will be published elsewhere.

Genome size analysis—Genome sizes (DNA contents) were measured for 22 lines (Table 2). All DNA values are expressed relative to the standard adjusted to 100 FAU. Leaf epidermal nuclei were chosen for measurement because of their consistency in size and stainability among lines (Price et al., 1980). These nuclei were of 4C DNA content as previously reported for corn by Swift (1950). Initially, 100 nuclei of Va35 were measured to obtain a mean and coefficient of variation (C.V.) for the standard corn epidermal nuclei. The mean was 96.20 ± 4.16 and the C.V. was 4.32. Therefore, to obtain a 90% probability of detecting a 5% difference in genome size between two plants ($\alpha = 0.05$), 30 nuclei per individual were measured (Gold, Bennett and Gall, 1975).

The average DNA content of all the corn lines examined was $104.23 \pm SD = 4.38$ FAU. The mean FAU among all lines ranged from 95.23 FAU in Gaspe Flint to 117.52 FAU in Zapalote Grande, a 23% difference in genome size. The range among inbreds was from 97.82 FAU in Ar206 to 113.39 FAU in Tx601, a 16% difference in genome size. Line Ar206 had nuclei that were morphologically inconsistent and was removed from further correlation analyses.

A nested analysis of variance indicated significant differences among lines. A Duncan's multiple range test indicates those lines that are significantly different from each other at $\alpha = 0.05$ level (Table 2).

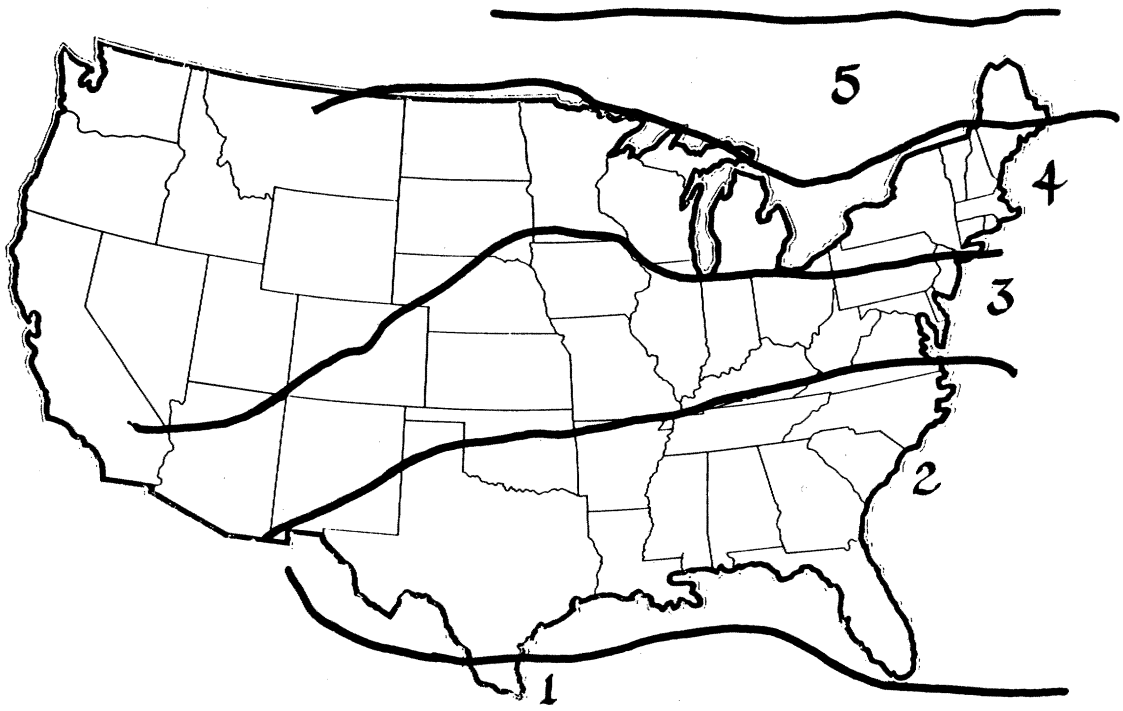

Fig. 1. Geographical maturity zones for North American *Zea mays* lines.

Correlation analyses—Pearson's correlation coefficients were calculated using the estimates of % Het, DNA content, and zones. Coefficients were calculated for all lines and for the inbreds with the open-pollinated varieties deleted. Among all lines, significant negative correlations were obtained between zone and C-band number, % Het, and genome size. Significant, positive correlations were obtained (as might have been expected) between C-band number and % Het and genome size, and between % Het and genome size (Table 3). When only the inbred lines were considered, significant positive correlations were found between C-band number and % Het and genome size, and between % Het and genome size (Table 4). Correlations between zone and C-band number, % Het and genome size were negative but nonsignificant (Table 4).

DISCUSSION—We have demonstrated a 23% variation in DNA content among varieties of *Zea mays* by microspectrophotometry. The lowest 4C DNA content was from Gaspe Flint (9.82 pg) and the highest was from Zapolate Grande (12.12 pg). Zapolate Grande had two B chromosomes. The overall range in 4C DNA content, excluding Zapolate Grande, was 19%.

Significant positive correlations of C-band number, the percent of the karyotype in C-band heterochromatin, and genome size were found among all maize lines and among inbred lines only. These correlations are consistent with reports of other genera. Heterochromatin amount and genome size have been positively

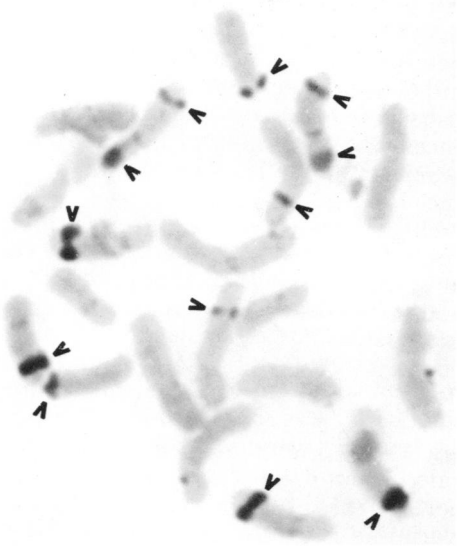

Fig. 2. A karyotype of *Zea mays*, variety F6. Twelve well-differentiated C-banded regions are indicated by arrows.

TABLE 2. *DNA content, percentage of C-band heterochromatin, and C-band number of corn lines*

Line	4C DNA content			Duncan's[c] groups			% Het	C-band number
	FAU	±SE	Picograms[b]					
ZG	117.52	0.65	12.12		A		16.2[d]	18[e]
Tx601	113.39	0.72	11.69		B		16.9	15[e]
Ky27	111.06	0.65	11.45		C		16.2	13[e]
Ky21	108.89	0.52	11.23		D		—	—
NT	108.78	0.59	11.22		D		15.6	14[e]
KYS	108.68	0.58	11.20		D		7.9	10
Ge281	106.83	0.56	11.01		E		12.7	16
NY16	105.64	0.60	10.89		E		14.2	14
Mo8w	104.02	0.60	10.72		F		7.2	8[e]
Tx5855	104.02	0.62	10.72		F		11.3	10
Ms116	103.19	0.54	10.64		F	G	8.6	7[e]
Pa88	101.90	0.67	10.51		H	G	9.1	10
Mp488	100.98	0.58	10.41	I	H		9.1	12[e]
Va35	100.00[a]	0.43	10.31	I			1.8	2
T8	99.87	0.47	10.30	I	J		3.1	3[e]
F6	99.81	0.79	10.29		J		8.7	12
Mo15w	99.35	0.55	10.24		J	K	6.4	7[e]
TKF	98.87	0.60	10.19		J	K	0	0
NY302	98.59	0.54	10.16		J	K	1.0	4
SD9	98.39	0.62	10.14		J	K	8.4	8
Ar206	97.82	0.71	10.08			K	11.7	12
GF	95.23	0.63	9.82		L		3.1	4

[a] The genome size of the standard, Va35, was defined as 100 FAU for this study.
[b] Picogram values are relative to Va35, which was calculated by Laurie and Bennett (1985) to have a 4C DNA content of 10.31 pg.
[c] Means with the same letter are not significantly different ($\alpha = 0.05$).
[d] ZG contained two B chromosomes, which were not added into the % C-band heterochromatin because of their non-C-banding phenotype. B chromosomes were detected in none of the other lines of this study.
[e] Plants were heterozygous for the presence and absence of at least one C-band.

correlated in several plant taxa including *Lolium* (Thomas, 1981), *Secale* (Bennett, Gustafson and Smith, 1977), and *Gibasis karwinskyana* (Kenton, 1983). Furthermore, there are ample reports from both animals (John and Miklos, 1979) and plants (Appels, Driscoll and Peacock, 1978; Bedbrook et al., 1980; Deumling and Greilhuber, 1982) that indicate a general association between highly repetitive DNA sequences and heterochromatin. In corn a highly repeated 185 base pair satellite DNA sequence has been shown to reside in knob and C-band heterochromatin (Peacock et al., 1981). Differential amounts of this sequence may be contributing to the variation in DNA content detected in the present study.

Positive correlations between increasing DNA content and increasing latitude have been reported for genera of grasses (Avdulov, 1931), comparisons of plant families (Levin and Funderburg, 1979), and among cereal grain crops, cultivated pasture grasses, and pulse crops (Bennett, 1976b). Within the genus *Secale,* a 20% variation in DNA content occurs, which is positively correlated with variation in the amount of C-band heterochromatin (Bennett et al., 1977). The *Secale* species that grow at higher latitudes or altitudes have higher DNA amounts than those growing at lower latitudes or altitudes (Bennett, 1976a).

Within *Zea mays,* the relationship of DNA content with latitude does not fit the above pattern observed among related species. Rather, DNA content is negatively correlated with increasing latitude. Supporting evidence for an inverse cline of DNA content and latitude comes from the distribution of DNA content determinations for corn lines from different latitudes reported by Laurie and Bennett (1985). The reasons for the pattern of distribution of DNA contents in *Zea mays* are speculative but may relate to selection pressures imposed by man. These selective pressures may influence DNA content via its nucleotypic effects. Among

TABLE 3. *Correlation coefficients matrix of C-band number, percent C-band heterochromatin, FAU, and zone using all corn lines*

	Band no.	% Het	FAU	Zone
Band no.	—	0.91[a]	0.74[a]	−0.52[a]
% Het		—	0.77[a]	−0.47[a]
FAU			—	−0.45[a]
Zone				—

[a] Significant at the 0.05 level.

TABLE 4. *Correlation coefficients matrix of C-band number, percent C-band heterochromatin, FAU, and zone using the inbred lines*

	Band no.	% Het	FAU	Zone
Band no.	—	0.87[a]	0.59[a]	−0.33
% Het		—	0.70[a]	−0.23
FAU			—	−0.12
Zone				—

[a] Significant at the 0.05 level.

herbaceous angiosperms, DNA content has been shown to correlate with several nucleotypic parameters including nuclear volume (Baetcke et al., 1967), cell volume (Price, Sparrow and Nauman, 1973), mitotic cycle time (Van't Hof, 1965; Evans and Rees, 1971), the duration of meiosis (Bennett, 1971), and minimum generation time (Bennett, 1972).

Bennett (1972) proposed that characteristics such as rapid mitotic cycle time, which would allow a plant to develop rapidly in a time-limited environment, require a low DNA content. Northern flint corns are adapted to cooler regions, more mesic soils, and a shorter growing season. Galinat and Gunnerson (1963) proposed that corn originated in Mexico and was taken north until environmental barriers (primarily a shorter growing season) prevented normal maturation. To overcome this limitation, the obvious adaptation would be more rapid maturation. In this study, the two northern flint corns examined had the smallest genomes. Gaspe Flint, which had the smallest genome, also has a very short interval from germination to flowering (35 days; D. L. Shaver, pers. comm.). Zapalote Grande has a large genome and the long maturation time typical of Mexican corn races (Wellhausen et al., 1952). Although no significant correlation between maturation zone and DNA amount was observed among the inbred lines, it is nevertheless interesting that SD9 with the smallest genome of the inbreds has a short interval from germination to flowering (ca. 65 days). In contrast, Tx601 with the highest DNA content of the inbreds has a longer interval to flowering (ca. 100 days; J. D. Smith, unpubl.).

Maize is somewhat unique among cultivated grasses with respect to both morphology and range of adaptation. It exists only as a cultivated crop, and both its morphology and the economics of primitive agriculture would have encouraged selection for higher-yielding individual plants. Within the constraints imposed by the length of the growing season, a general relationship between individual plant size and yield has long been observed in maize. Thus, it is quite reasonable to suspect that the northward migration of corn as a cultivated species was accompanied by selection for earlier flowering and selection for maximum plant size permitted by climatic constraints.

If such speculations are correct, the simultaneous selection by man for earlier maturation and large plants may be related to the lower DNA content of corn varieties adapted to higher latitudes. For example, in Gaspe Flint, flowering occurs at about 35 days after germination, and most growth after that results from enlargement of preexisting cells. Therefore, selection for larger plants may have been achieved through selection for more cells. More cells could result from the shorter mitotic cycle time that correlates with reduced DNA amounts.

The positive correlations reported for DNA content and latitude in other plant species and the negative correlation observed for corn indicate that alternative strategies for adaptation to cooler climates and shorter growing seasons exist. Among the plant families, genera, and species for which DNA clines have been detected, artificial selection for individual plant yield apparently has accompanied selection for climatic adaptation only in *Zea mays*. This suggests that the inverse cline observed for maize relates to man's intervention.

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