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Genome size variation in *Zea mays* ssp. *mays* adapted to different altitudes

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Summary. Previous studies have indicated a positive correlation between genome size and altitude among plant species. It has been hypothesized that increasing genome size occurs due to increasing C-banded heterochromatin. In corn, increasing altitude has been correlated with decreasing knob (C-banded) heterochromatin, suggesting that DNA content may decrease with increasing altitude. In this study, nuclear DNA content of 12 southwestern United States Indian maize populations, collected at various altitudes, was determined. The significant positive correlation observed between genome size and altitude suggests that corn follows the trend of increasing DNA content with increasing altitude observed in other plant species. Whether this correlation is due to increasing knob heterochromatin or additional intra- or supernumerary chromosomal DNA sequences has yet to be determined.

Key words: Genome size – Corn – Flow cytometry

Introduction

As high as a 38.8% variation in genome size in *Zea mays* ssp. *mays* has been reported (Laurie and Bennett 1985; Rayburn et al. 1985). Corn lines from the higher latitudes of North America have significantly lower nuclear DNA amounts than those of lower latitudes (Rayburn et al. 1985). These results were corroborated by findings of Laurie and Bennett (1985), and agree with previously published reports on heterochromatic knob distribution in maize.

McClintock (1929) first reported the existence of large heterochromatic segments on corn pachytene chromosomes called knobs. Since that time, numerous studies have attempted to correlate knob distribution with various characteristics. In corn types grown by North American Indians, knobs are prevalent in the southwestern group and are rare in the northern group (Longley 1938). Brown (1949) observed that corn lines from the northern United States had fewer knobs than corn types from the southern U.S., and that an apparent gradation of knobs exists north to south. Rayburn et al. (1985) noted that the number of positive staining C-bands are also negatively correlated with latitude. Positive C-bands in mitotic chromosomes are apparently pachytene knobs (Hadlaczky and Kalman 1975; Ward 1980). Rayburn et al. (1985) also observed a significant positive correlation between band number (knob number) and 4C DNA content, thereby indicating that the negative correlation between knob number and latitude reflects the correlation between genome size and latitude.

Mangelsdorf and Cameron (1942) observed a linear correlation between knob number and altitude in Guatemalan corn lines. Lines from higher altitude had fewer knobs than those from lower elevations. Longley and Kato (1965) observed a similar correlation. Bennett (1976b), upon re-evaluating the data of Wellhausen et al. (1952), corroborated the phenomenon in Mexican corn lines. If the correlation between knob number and DNA content were to hold in these studies, one would expect the corn lines adapted to higher altitudes to have lower nuclear DNA amounts than lines from lower altitudes. This correlation is in contrast to published reports in other species.

In the genus *Secale*, adaptation to higher elevation appears to be correlated with increasing DNA amounts (Bennett 1976a; Smith et al. 1976). This same correlation (higher DNA amounts at higher elevations) has also been observed in teosinte, the closest wild relative of *Zea mays* ssp. *mays* (Laurie and Bennett 1985). Due to the appar-

ent conflict with existing genome size studies and to the imprecise determinations of altitudes of the various corn lines studied, the exact relationship between genome size and altitude in corn has yet to be determined. The purpose of this study is to provide direct evidence of the adaptation of *Zea mays* ssp. *mays* populations to various altitudes.

Materials and methods

The corn populations selected for this study (Table 1) are open-pollinated southwestern United States Indian corn populations that were obtained from the North Central Regional Plant Introduction Station at Ames/IA. They were selected as types typically grown and initially collected at the elevations indicated (Table 1). The origin of these populations was restricted to the state of Arizona (USA) to avoid wide ranges of latitude and/or longitude. The total range of altitude was 5,200 feet. In addition, the lines were selected on the basis of their description, in order to minimize the possibility of selecting accessions collected from the same or very similar populations.

The nuclear DNA contents of the various corn lines were determined according to Rayburn et al. 1989. Nuclei were isolated from 2-week-old seedlings. Rayburn et al. (1989) demonstrated that flow cytometry could be used to detect significant genome size variation among corn lines. They found that the fluorochrome DAPI was essential to accurately determine the precise variation among individuals in corn. Due to the importance of properly titrating the dye to DNA ratio with this fluorochrome, an inbred line 'Va35' was used as an external standard, rather than using it as an internal standard and resulting in a heterogeneous sample that could make the titration rather difficult. Rayburn et al. (1989) have used this method in corn and found that it compares favorably with microdensitometry results.

Table 1. Southwestern Indian maize populations observed in this study, location of origin, and altitude

P.I. no.[a]	Location collected[b]	Altitude	Description
218161	Navajo Indian reservation near Shonto trading post	5,300 ft	Yellow flour corn
218162	Navajo Indian reservation near Shonto trading post	5,300 ft	Mixed-color flour corn
218163	Navajo Indian reservation near Shonto trading post	5,300 ft	White flour corn
218174	Moencopi Pueblo	4,300 ft	Sweet corn
218175	Moencopi Pueblo	4,300 ft	Blue flour corn
218177	Moencopi Pueblo	4,300 ft	Pink flour corn
218179	San Xavier del Bac	2,300 ft	Cream-color flour corn
218180	San Xavier del Bac	2,300 ft	
218181	Papago reservation near San Xavier del Bac	2,300 ft	Used for cattle feed
218186	Mojave Indian reservation near Parker	300 ft	Used for boiling and for cornmeal
218187	Mojave Indian reservation near Parker	300 ft	Cream-white flour corn used for parching and cornmeal
218189	Sommerton	100 ft	White dent corn

[a] North Central Plant Introduction Station accession number
[b] All accessions are from the state of Arizona

While Rayburn et al. (1989) determined genome size on an individual plant basis, flow cytometric analysis allows one to pool individuals in order to determine the mean nuclear DNA content of the population. Since, statistically, both methods should result in similar estimates of the mean, one may take advantage of the flow cytometer's ability to examine large numbers of nuclei to estimate the population mean. Due to mechanical problems with releasing nuclei from a large number of individuals at one time and to minimize ambiguous results due to isolation or staining irregularities, two samples consisting of three plants per sample were analyzed. In addition, after the nuclei were isolated, the samples were divided in half and each half was stained and analyzed separately. This was to minimize variability due to improper titration of the dye to nuclei ratio.

Using this method, inbred lines have been observed to have a single G_1 and G_2 peak. The coefficient of variation (CV) of the G_1 peaks has been between 3 and 4 (Rayburn et al. 1989). Preliminary results have demonstrated that when three plants of an inbred line are combined, the same peaks appear with CVs remaining the same (L. M. McMurphy and A. L. Rayburn, unpublished results). These results indicate (1) the homogeneity of the nuclei isolated, and (2) the validity of pooling plants without affecting the estimates of the mean and/or CV of a population. Additional G_1 peaks observed and/or increase in CVs, therefore, indicate plant heterogeneity within the population.

The two nuclei samples of each line were analyzed on a Coulter EPICS 751 flow cytometer-cell sorter system. The excitation beam was provided by a 5-W argon laser. The laser was tuned to an excitation wavelength band of 351–365 nm. A minimum of 5,000 nuclei were examined for each sample. Each day the flow cytometer-cell sorter was calibrated with the standard inbred corn line 'Va35'. The fluorescence intensity of 'Va35' was set at fluorescence channel 100. The mean DNA content was expressed as mean fluorescence channel relative to 'Va35'. This figure was defined as DNA arbitrary unit (AU). 'Va35' was defined as having a nuclear DNA content of 100 AU.

Significance of genome size variation among these lines was determined from a nested analysis of variance. Linear correlation analyses were run to determine if a relationship between genome size and altitude exists.

Results

A summary of the mean nuclear DNA content per population is given in Table 2. The range of variation was 29.3% among the 12 lines studied. All lines were observed to a have a nuclear DNA content higher than 'Va35'. The coefficient of variation (CV) for three plants of 'Va35' was 4.0–4.5, while the CVs of the experimental populations ranged from 4.5 to 6.5. In one population, P.I. 218186, the histogram obtained from the flow cytometric analysis indicated two distinct G_1 peaks (Fig. 1). The means of the two individual G_1 peaks were 114 and 133.8 AU. The CVs of the two peaks were $\approx$4.4 for the 114 AU peak and $\approx$3.7 for the 133.8 AU peak. Of the three plants in the sample, two appeared to fall in the

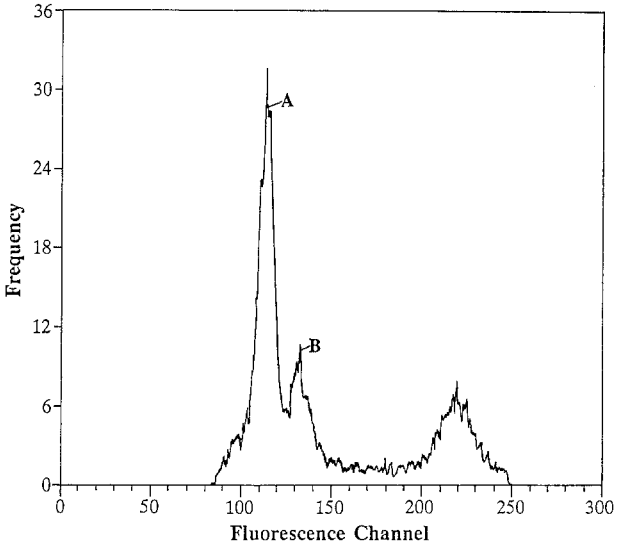

Fig. 1. Histogram of nuclei distribution with respect to DNA content in corn accession P.I. 218186. *A* and *B* denote the two distinct G_1 peaks observed

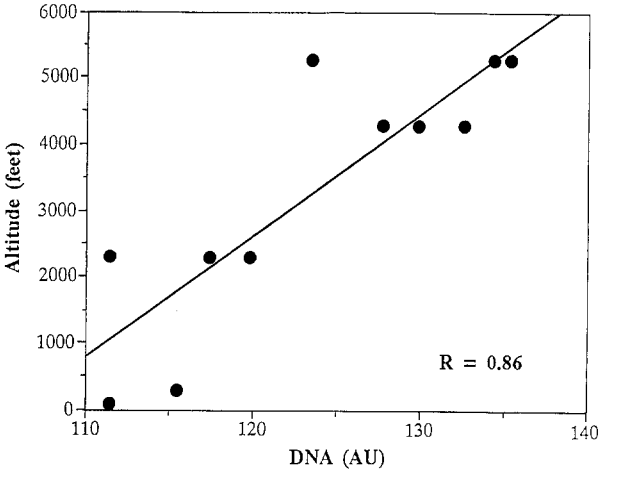

Fig. 2. Distribution of genome size with respect to altitude in the 11 corn populations analysed

lower of the two peaks, indicating lower DNA content. Since this was the only line observed to have this phenomenon, it was removed from statistical analyses.

A nested analysis of variance was performed in the General Linear Model (GLM) procedure of pc SAS. The GLM procedure was used due to an unbalanced data set caused by a limited seed supply of two populations, P.I.s 218161 and 218174. This resulted in only one sample (three plants) examined for these accessions. The analysis indicated highly significant differences among populations. Significance was also noted between the two separate nuclei isolations within a population, however, the amount of variation was small relative to the among-population variation. A Duncan's multiple range test

Table 2. Nuclear DNA content of the 11 populations in the statistical analyses

P.I. no.	Mean nuclear[a] DNA content	Standard deviation	Picograms per 4C nucleus[b]	Duncans grouping[c]
218163	135.2	1.3	13.5	A
218162	134.2	3.8	13.4	A
218177	132.4	0.7	13.2	A B
218174	129.7	0.6	13.0	A B
218175	127.5	1.5	12.8	A B C
218161	123.2	2.3	12.3	C D
218180	119.7	3.7	12.0	D E
218179	117.2	1.0	11.7	D E F
218187	115.5	0.7	11.6	E F
218189	111.4	1.6	11.1	F
218181	111.3	2.2	11.1	F

[a] Means expressed in DNA arbitrary units (AU) relative to Va35 = 100 AU
[b] Based on Va35 = 10.03 pg (Rayburn et al. 1989)
[c] Means with same letter are not significantly different at the $\alpha = 0.05$ level

indicated which populations were significantly different at the $\alpha = 0.05$ level (Table 2).

A linear correlation analysis indicated a significant, positive correlation at the $a = 0.05$ level (Fig. 2). The r value was 0.86. Observing the distribution of genome size with respect to altitude indicates the occurrence of two clusters. Populations at 2,300 ft elevation and below tended to cluster at lower DNA content level than those at 4,300 ft and above. Due to the lack of populations from altitudes between 2,300 ft and 4,300 ft, it was unclear as to whether the observed correlation was due to continuous or discontinuous distribution.

Discussion

All of the corn populations had a significantly higher nuclear DNA content than the standard inbred line. Longley (1938) observed that the Indian maize strains from Arizona and New Mexico had an average of 9.5 knobs per plant. 'Va35', the standard inbred used in this study, has been observed to have one knob (Rayburn et al. 1985). If knob number and genome size are positively correlated, one would expect the Arizona populations used in this study to have a relatively large genome size compared to 'Va35'.

The higher CVs observed in the Indian populations appear to be due to the heterogeneous nature of the populations. 'Va35' appears to be homogeneous for genome size. The ≈4.0 CVs observed in this study were similar to the CVs reported previously for 'Va35' (Rayburn et al. 1989). Since three plants were combined in the present study while individual plants were examined in the previous study, there appears to be little if any plant-

to-plant variation with regard to genome size in 'Va35'. One would expect that populations heterogeneous for genome size and sampled as in the present study would result in slightly higher CVs.

Several of the populations examined in this study were observed to be heterogeneous for a number of characteristics. Six of the populations segregated for kernel color. In most of the lines, the seedlings germinated and/or emerged over a period of several days as opposed to 'Va35', which germinated uniformly. The plants chosen for genome size determinations were selected at random in order to obtain unbiased data. That the populations could also be heterogeneous with respect to genome size is apparent from Fig. 1. P.I. 218186 was observed to have plants of significant differences in genome size resulting in two G_1 peaks on the data analysis histogram. If the remaining populations observed in this study were heterogeneous for genome size but did not vary to the extent of P.I. 218186, the histogram might show a broadening of the G_1 peak and an increased CV instead of appearing as two distinct peaks. The varietal collections might be expected to be heterogeneous, whereas 'Va35' is a uniform inbred line that has been inbred for at least 20 generations.

The observed positive correlation between genome size and altitude is similar to that observed among other plant species. Within the genus *Secale*, those species that are found at the higher altitudes have larger nuclear DNA amounts than those at lower altitude (Bennett 1976a). Smith et al. (1976) estimated that 50%–75% of the variation in DNA amount was accounted for by positively stained C-banded heterochromatic regions along the chromosome. Bennett (1976b), therefore, concluded that adaptation to higher elevation was associated with an increase in DNA amount due to increasing amounts of heterochromatin.

Increasing genome size may not be correlated with increasing C-banded heterochromatin in corn. Positive C-bands in corn are the visualization of heterochromatic knobs at mitosis. Published reports with respect to knob number variation and altitude have indicated an inverse correlation between knob number and altitude (Longley 1938; Mangelsdorf and Cameron 1942). If genome size is positively correlated to knob number and/or amount of positive C-banding heterochromatin, one would expect that lines collected at high altitudes would have a lower DNA content than those collected at lower altitudes. In this study, however, genome size was observed to increase with increasing altitude. Two possible hypotheses as to these results are: (1) in the populations examined in this study, knob number is not negatively correlated with altitude, or (2) major changes in genome size can be achieved in corn by amplification or deletion of DNA sequences other than those found in the positively staining knob regions.

No detailed study to date has examined the question of knob number versus altitude in the southwestern U.S. Indian populations used in this study. These maize populations have been demonstrated to be similar to the Mexican corn races (Doebley et al. 1986). Bennett (1976b) observed the negative correlation between altitude and knob number in the Mexican races. There are, therefore, no indications that the negative correlation should not hold for the southwestern maize.

Rivin et al. (1986) have demonstrated that repeated DNA sequences other than the knob sequence may fluctuate dramatically in copy number in corn. In addition, Phillips (1978) reported variation in the rRNA gene multiplicity among 20 inbred lines of corn. Although Phillips did not report a correlation between DNA amount and rRNA gene copy number, one might expect that larger rRNA gene fluctuations could result in variation in genome size. The preceding studies indicate that intrachromosomal DNA variation other than knob sequences could influence DNA amounts.

Alternatively, supernumerary chromosomal DNA changes could also be involved. *B* chromosomes represent such changes. *B* chromosomes are defined on the bases of two criteria, distribution within a population and pairing behavior at meiosis (Jones and Rees 1982). *B* chromosomes are not found in all individuals of a given population. Since normal growth and reproduction occur in the absence of the *B* chromosomes, indications are that *B* chromosomes are dispensable. In meiosis, *B* chromosomes pair with one another and not with members of the normal (*A*) chromosome complement. They are, therefore, not homologous with the *A* chromosomes. The *B* chromosome of corn is a subtelocentric chromosome that is observed to have large blocks of heterochromatin at pachytene. Interestingly, when C-banding techniques are used to reveal constitutive heterochromatin in mitosis, the *B* chromosomes do not demonstrate the large positive-staining regions expected (Rayburn et al. 1985; Ward 1980). Due to nondisjunction at the second pollen mitosis, the number of *B* chromosomes can fluctuate in a population (Carlson 1978). Jones and Rees (1982) have estimated the amount of DNA present in a *B* chromosome to be 0.5 pg. Variation in the number of *B* chromosomes from plant to plant would result in major changes in nuclear DNA amounts.

Longley (1938) observed that certain southwestern Indian maize contained *B* chromosomes. In addition, Longley observed that *B* chromosomes were more prevalent in plants with low knob number than plants with high knob number. One possible hypothesis as to the results of this study is that knob number may indeed be decreasing with increasing altitude, but the number of *B* chromosomes may be increasing.

In summary, a significant positive correlation was observed between altitude and genome size in *Zea mays*

ssp. *mays*. This correlation is in agreement with the trends observed in other plant species. That the correlation seems in direct opposition to the trend of decreasing knob number with increasing altitude may be due to: (1) a positive correlation between knob number and altitude in these populations, (2) intrachromosomal DNA variation not involving the knob sequence, or (3) supernumerary chromosomal variation. Further investigations are underway to determine which DNA sequences may be increasing with increasing altitude.

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