Acta Bot. Neerl. 39(2), June 1990, p. 197–202

Nuclear DNA content variation in the ancient indigenous races of Mexican maize

A. L. RAYBURN and J. A. AUGER[1]

Department of Agronomy and [1]The Cell Sciences Laboratory of the Biotechnology Center, Turner Hall, University of Illinois at Urbana-Champaign, Urbana, Illinois, U.S.A.

SUMMARY

From eight maize accessions representing the four ancient indigenous races of mexican maize the nuclear DNA content was determined by flow cytometry. No significant difference between accessions of the same race was observed. Significant differences were found among the races, with two clusters observed. The lowland races had an average genome size of 12·6 pg while the highland races showed an average genome size of 11·1 pg. A significant negative correlation between genome size and altitude was observed.

Key-words: genome size, adaptation, altitude, corn, nuclei.

INTRODUCTION

The ancient indigenous races have been described taxonomically as the most primitive of the Mexican maizes (Doebley 1983; Wellhausen *et al.* 1952). The four distinct races that comprise the group are Palomero Toluqueno, Arrocillo Amarillo, Chapalote and Nal-Tel (Wellhausen *et al.* 1952). All of the races have small ears, are relatively early in maturity and are pop corns. Palomero Toluqueno and Arrocillo Amarillo adapted to high elevations while Chapalote and Nal-Tel appear adapted to tropical low elevations. The average knob number as reported by Wellhausen *et al.* (1952) for each race is 1·2 knobs for Palomero Toluqueno, 6 for Chapalote and 5·5 for Nal-Tel. Whereas the average knob number of Arrocillo Amarillo was not determined in the previous study, McClintock *et al.* (1981) indicated that the average knob number may be low.

Laurie & Bennett (1985) reported the DNA content of three of the four ancient indigenous races. The 4C nuclear DNA amount reported for Chapalote was 11·65 pg, for Palomero Toluqueno 11·27 pg and for Nal-Tel 11·92 pg. Rayburn *et al.* (1985) reported a 4C genome size of 11·22 for Nal-Tel. That the genome sizes of these races appear to vary only slightly is surprising considering the differences in knob number among the populations. Rayburn *et al.* (1985) have demonstrated a positive correlation between knob number and genome size. Recent studies have also implicated the geographical habitat as having an effect on genome size (Bennett 1976a; Laurie and Bennett 1985; Rayburn *et al.* 1985, 1990).

The purpose of this study was to re-evaluate the genome size variation among these four maize races, in order to ascertain (1) whether genome size is related with knob number and altitude and (2) whether genome size may be indicative of phylogenetic relationships.

Correspondence: Dr A. Lane Rayburn, Department of Agronomy, Turner Hall, 1102 South Goodwin Avenue, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, U.S.A.

Table 1. Ancient indigenous races of Mexican maize used in the present study

Race	ID No.[1]	Altitude (m)	Mean 4C nuclear DNA amount (AU)	SD
Arrocillo Amarillo	1212	—	111·2	3·4
Arrocillo Amarillo	1428	2060	110·5	3·0
Chapalote	861	61	126·1	3·9
Chapalote	10 509	75	129·7	5·8
Nal-Tel	69	300	124·2	4·0
Nal-Tel	815	30	123·7	0·7
Palomero Toluqueno	2233	2652	113·7	0·1
Palomero Toluqueno	2234	2652	108·7	8·3

[1]CIMMYT identification number.

MATERIALS AND METHODS

The maize accessions selected for this study represent the four ancient-indigenous races of Mexican maize as described by Wellhausen *et al.* (1952). Two accessions from each race were obtained from Dr Taba of CIMMYT and are listed in Table 1.

The nuclear DNA amounts of the corn accessions were determined according to Rayburn *et al.* (1990). Nuclei were isolated from three seedlings. For each accession, two nuclear isolations were performed, resulting in six plants per accession analysed. The DNA fluorochrome DAPI was used to stain the nuclei.

The nuclear samples were analysed on a Coulter EPICS 751 flow cytometer–cell sorter system. The excitation beam was provided by a 5 W argon laser tuned to an excitation wavelength of 351–365 nm. The power of the output beam was 100 mW. Five thousand nuclei were examined for each sample. The flow cytometer was calibrated daily. The fluorescence intensity of Va35 (a well-characterized maize inbred line) was used as a standard and was set at channel 100. This value was defined as 100 Arbitrary Units (AU). Arbitrary units were converted to picograms on the basis of 100 AU = 10·03 pg as determined by the standard inbred line Va35 (Rayburn *et al.* 1990).

Significance of genome size variation among the lines was determined from a nested analysis of variance. Statistical analyses were also performed to determine possible relationships between altitude and/or evolutionary divergence and genome size.

RESULTS

In all the nuclei isolations sampled, only two peaks were observed in each histogram (Fig. 1). The coefficient of variation (CV) observed for each sample was similar to the CVs observed in published reports of flow cytometric analyses in plant species (De Laat *et al.* 1987; Rayburn *et al.* 1990). Due to the similarities of the CVs, it would appear that the plants within each isolation were homogeneous with respect to genome size. The experiments as described above, given the CV of 4·0 among sample means, would have a 75% probability of detecting a real difference of 11% among sample means at the $P < 0{\cdot}10$ level (Gold *et al.* 1975). The mean nuclear DNA contents per accession are listed in Table 1.

A nested analysis of variance was performed in the General Linear Model (GLM) procedure of PCSAS. The analyses indicated no significant differences among different

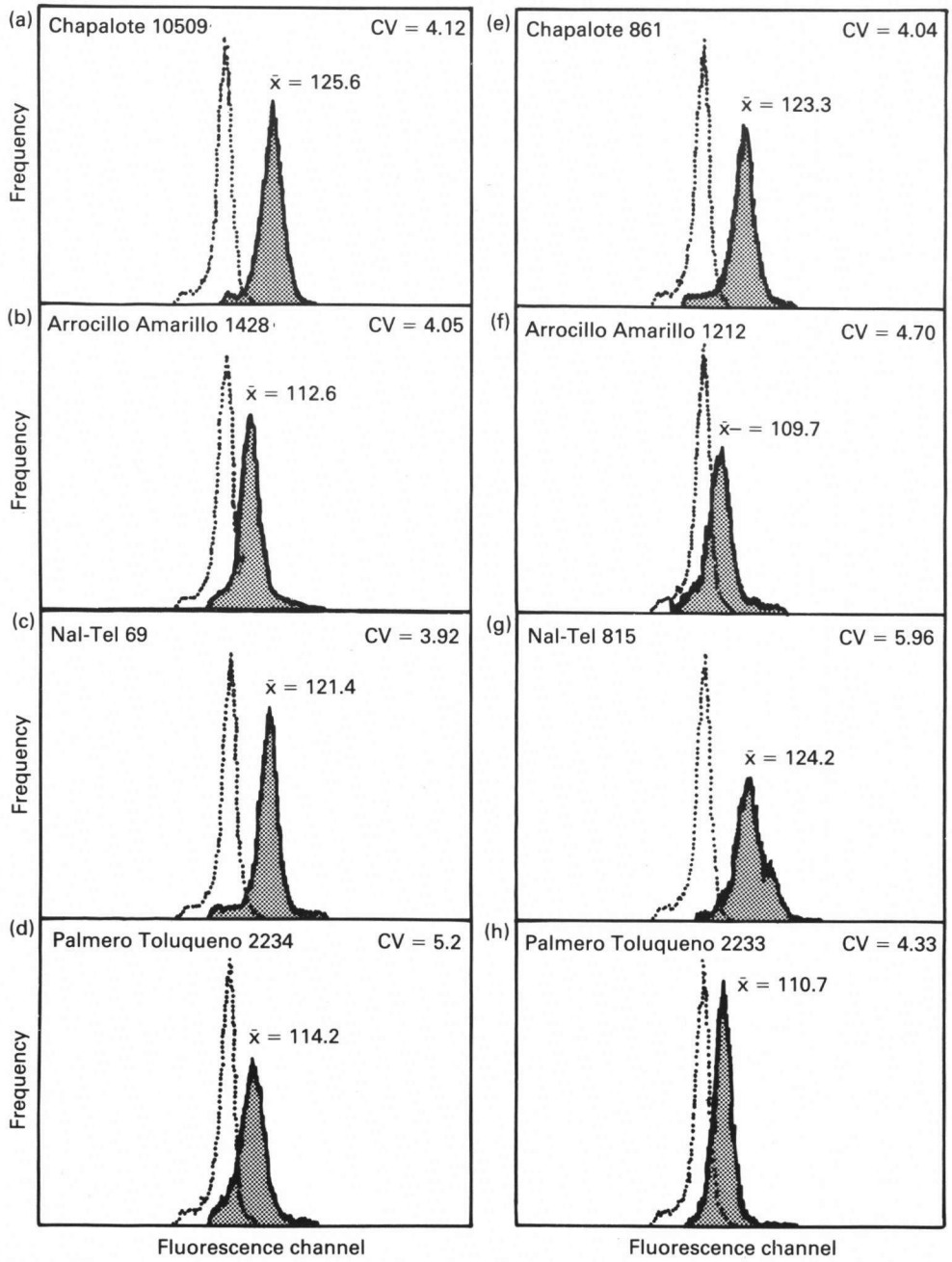

Fig. 1. Histograms representing the distribution of nuclei from a single nuclear isolation of each accession analysed. (– – –) Represents Va35, the external standard, and is set at channel 100. The shaded peaks represent the G_1 peaks observed for each accession.

accessions of the same race. A highly significant difference was observed among the four races. A Duncan's range test indicated which races were significantly different at the $P < 0{\cdot}05$ level (Table 2).

Table 2. Statistical analyses of genome size with respect to race

Race	Mean 4C nuclear DNA content (pg)	CV	Duncan's grouping[1]
Arrocillo Amarillo	11·0	2·4	A A
Palomero Toluqueno	11·2	5·0	A
Nal-Tel	12·4	1·9	B B
Chapalote	12·8	4·3	B

[1]Means with the same letter are not significantly different at the $P<0.05$ level.

A linear correlation analysis indicated a significant negative correlation at the $P<0{\cdot}05$ level between genome size and altitude. The r value was 0·94. The distribution of genome size with respect to altitude resulted in the appearance of two clusters. Palomero Toluqueno and Arrocillo Amarillo were clustered around an average mean DNA content of 111 AU while Nal-Tel and Chapalote were clustered around an average of 123 AU.

DISCUSSION

In contrast to Laurie & Bennett (1985), the mean number DNA contents of Chapalote and Nal-Tel were significantly different from Palomero Toluqueno. Upon converting the AU reported into pg, the DNA values for Palomero Toluqueno (11·25 pg, Laurie & Bennett, 1985; 11·2 pg, present study) compare quite well. However, in this study the DNA values of 12·4 pg and 12·8 pg for Nal-Tel and Chapalote respectively, were significantly higher than the 11·92 pg and 11·65 pg reported by Laurie & Bennett (1985). In addition, the estimate of 12·8 pg for Nal-Tel was also considerably higher than the 11·22 pg reported by Rayburn *et al.* (1985). Potential hypotheses to these conflicting results include presence of B chromosomes, heterogeneity of knob loci or fluctuations of additional DNA sequences. Studies are now underway to determine which of these hypotheses may be correct.

The mean nuclear DNA content appeared to be correlated with the knob number of the accession. Chapalote and Nal-Tel, having an average of 6 and 5·5 knobs respectively, might be expected to have similar genome sizes if knob number and genome size are correlated, as suggested by Rayburn *et al.* (1985). With the DNA values of 12·4 pg for Nal-Tel and 12·8 pg for Chapalote, this was the case. In addition, with the remaining two races having low average knob numbers (1·2 for Palomero Toluqueno and <3 possibly for Arrocillo Amarillo), one might expect these races to have a similar genome size and for their genome sizes to be smaller than either Chapalote and Nal-Tel. The observation of 11·2 pg for Palomero Toluqueno and 11·0 pg for Arrocilla Amarillo followed these expectations. Therefore, the results of this study demonstrate the positive correlation between knob number and genome size.

The significant negative correlation of altitude and genome size observed in this study (Tables 1 and 2) is in direct contrast with the correlation among crop plant species (Bennett 1976a,b). The Mexican races examined represented extremes in elevation. That these lines do not represent a range of altitudes, but only extremes, weakens the observed significance of the correlation between genome size and altitude. However, the genome

size data do appear to be compatible with the existing knob literature. Mangelsdorf and Cameron (1942), Longley and Kato (1965) and McClintock *et al.* (1981) observed that knob number was negatively correlated with altitude. Given the positive correlation between knob number and genome size, one would predict that the genome size of maize lines from high altitudes to be smaller than those from low altitude maize.

Mangelsdorf (1974) described six lineages which represented direct descendants from a proposed progenitor wild maize. Included in the extant ancestral species were three of the Mexican races examined here—Palomero Toluqueno, Chapalote and Nal-Tel. Primitive forms of Nal-Tel have been radio-carbon dated to 2500 BC, while cob remains, which have been identified as precursors of Chapalote, have been dated to 2300 B.C. It appears, therefore, that these races may represent early domestication of primitive maize. The large genomes of Chapalote and Nal-Tel could be a remnant of the ancestral genome of maize. Reduction in genome size has been correlated to specialization in certain species (Hinegardner and Rosen 1972).

Isozyme and chromosomal karyotypic analysis have resulted in a different racial clustering than that proposed by Wellhausen *et al.* (1952). Many of these systematic studies have resulted in division among races along elevations of adaptation (Bretting & Goodman, 1989; Doebley *et al.* 1985). Palomero Toluqueno and Arrocillo Amarillo are observed to have similar genomes sizes and appear to cluster as suggested by the isozyme data of Doebley *et al.* (1985). In contrast to Doebley *et al.* (1985), Nal-Tel and Chapalote were observed to have similar genome sizes. In the chromosomal analyses of Bretting & Goodman (1989), Nal-Tel and Chapalote clustered together while Palomero Toluqueno and Arrocillo Amarillo did not. All of the characteristics used in these studies (isozymes, chromosomes and genome size) appear to be correlated to altitude. Further analyses of additional Mexican races are under way to determine the racial clusters with respect to genome size and how this clustering relates to that described by Goodman & Brown (1988).

In summary, the average genome sizes of each of the four Mexican ancient indigenous races were re-evaluated. Contrary to previously published reports, genome size variation did exist. The two races adapted to lower elevations were observed to have significantly higher nuclear DNA content than those adapted to higher elevations. This trend agrees with the published chromosomal literature, but is in contrast to the genome size distribution observed among crop species in general. Due to the antiquity of the two lowland races, it is suggested that the large genome size may be a primitive characteristic of maize. Further investigations are under way to determine if genome size distributions in the Mexican races are correlated to published racial clusters.

REFERENCES

Bennett, M.D. (1976a): DNA amount, latitude, and crop plant distribution. *Environ. Exp. Bot.* **16:** 93–108.

— (1976b): DNA amount, latitude and crop plant distribution. In: Jones, K. and Brandham, P.E. (eds): *Current Chromosome Research.* Elsevier/North Holland Biomed. Press, Amsterdam.

Bretting, P.K. & Goodman, M.M. (1989): Karyotypic variation in mesoamerican races of maize and its systematic significance. *Econ. Bot.* **43:** 107–124.

De Laat, A.M.M., Gohde, W. & Volgelzang, M.J.D.C. (1987): Determination of ploidy of single plants and plant populations by flow cytometry. *Plnt. Brd.* **99:** 303–307.

Doebley, J.F. (1983): The maize and teosinte male influencence: a numerical taxonomic study. *Ann. Missouri Bot. Gard.* **70:** 32–70.

—, Goodman, M.M. & Stuber, C.W. (1985): Isozyme variation in the races of maize from Mexico. *Am. J. Bot.* **72:** 629–639.

Gold, J.R., Bennett, L.F. & Gall, G.A.E. (1975): A set of tables for determining minimum sample sizes necessary to show statistically significant differences among treatment groups using analysis of variance. University of California Special Publication 3051.

Goodman, M.M. & Brown, W.L. (1988): Races of corn. In: Sprague, E. S. and Dudley, J.D. (eds): *Corn and Corn Improvements.* ASA monograph, Academic Press Inc., New York.

Hinegardner, R. & Rosen, D.E. (1972): Cellular DNA content and the evolution of teleostean fishes. *Am. Nat.* **106:** 621–644.

Laurie, D.A. & Bennett, M.D. (1985): Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation. *Heredity* **55:** 307–313.

Longley, A.E. & Kato, T.A.Y. (1965): *Chromosome Morphology of Certain Races of Maize in Latin America.* Mexico Research Bulletin 1. International Center for the Improvement of Maize and Wheat, Chapingo, State of Mexico.

McClintock, B., Kato, Y.T.A. & Blumenschein, A. (1981): *Chromosome Constitution of Races of Maize.* Colegio de Postgraduados, Chapingo, Mexico.

Mangelsdorf, P.C. (1974): *Corn, its Origin, Evolution, and Improvement.* Harvard University Press, Cambridge, U.S.A.

Mangeldorf, P.C. & Cameron, J.W. (1942): *Western Guatemala: a secondary center of Origin of Cultivated Maize Varieties.* Harvard University Bulletin 1. Botany Museum.

Rayburn, A.L., Price, H.J., Smith, J.D. & Gold, J.R. (1985): C-Band heterochromatin and DNA content in *Zea mays*. *Am. J. Bot.* **72:** 1610–1617.

—, Auger, J.A., Benziger, E.H. & Hepburn, A.G. (1990): Detection of intraspecific DNA content variation in *Zea mays* ssp. *mays* by flow cytometry. *J. Exp. Bot.* **40:** (in press.)

Wellhausen, E.J., Roberts, L.M. & Hernandez, E.X. (1952): *Races of Maize in Mexico: Their Origin, Characteristics and Distribution.* Harvard University Press, Cambridge, U.S.A.