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Genome Size and Environmental Correlations in Maize (*Zea mays* ssp. *mays*, Poaceae)

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Variation in the DNA content attributed to sources such as variation in the amount of heterochromatin and occurrence of supernumerary chromosomes (Bs) in native races of maize cultivated at different altitudes (80–3620 m) is discussed. These populations present intra- and interpopulational variation in the DNA content of the regular complement (A-DNA) and the heterochromatic zones (DAPI bands). The mean number of Bs varied from 0 to 2·62 per plant among these populations, showing a positive correlation with the altitude of cultivation. In contrast, both the A-DNA content and the mean number of DAPI bands per plant were negatively correlated with altitude and the mean number of Bs per plant. These clinal variations in A-DNA content and the mean number of DAPI bands, and the inverse correlation of the mean number of Bs per plant over an altitudinal gradient could have an adaptative significance. Analysis of total DNA content and the number of DAPI bands, in individuals with different doses of Bs, indicates that in populations with high A-DNA content the increase in genome size due to Bs could be masked. This phenomenon is associated with the fact that individuals with Bs have a low number of DAPI bands. These results suggest that there is an optimum nucleotype for each population and that Bs are tolerated so long as this nucleotype is not exceeded.

Key words: Genome size, DNA content, environmental correlations, maize populations, heterochromatic bands, B chromosomes, *Zea mays* ssp. *mays*.

INTRODUCTION

The nucleotype is defined as the total nuclear DNA content (genic and non-genic) and it influences several cellular and developmental parameters such as chromosome size, nuclear volume, cellular volume, mitotic cycle time, duration of meiosis and minimum generation time (Bennett, 1987; Grant, 1987; Price, 1988*a*, *b*). Wide variation in nuclear DNA content has been described in a number of species and there are many examples of intra- and interspecific variation in genome size in the literature (Bennett and Smith, 1976, 1991; Price, 1988*a*; Poggio and Naranjo, 1990; Cavallini and Natali, 1991; Furuta and Nishikawa, 1991; Bennett and Leitch, 1995, 1997). Common causes of variation in DNA content include: polyploidy, aneuploidy, variation in the amount of heterochromatin, occurrence of supernumerary chromosomes (B chromosomes), deletion or duplication of chromosome segments, and variation in the copy number of repeated sequences. Rapid genome changes can also occur under stressful conditions (physical, chemical or genetic) (Walbot and Cullis, 1985). In this way, DNA could be unstable through the amplification or deletion of sequences which alter DNA content (Walbot and Cullis, 1985; Flavell, 1986; Cullis, 1990). Rapid genome changes that take place under stressful conditions are well documented in a variety of plants such as *Linum usitatissimum* (Cullis and Cleary, 1986; Cullis, 1990), *Microseris* (Price, 1988*a*), *Helianthus annuus* (Cavallini *et al.*, 1986; Natali *et al.*, 1993; Johnston *et al.*, 1996; Price and Johnston, 1996) and *Nicotiana* (Gerstel and Burns, 1966).

Rayburn *et al.* (1993) found instability in nuclear DNA content of F_1 hybrids of maize in several specific parental combinations. They found that the DNA amount of these hybrids was significantly higher than their respective parents, and they suggested that stability or instability of DNA sequence copy number depends on the parental inbred line used in each cross. Biradar and Rayburn (1993) found a relationship between the DNA content of these F_1 hybrids and heterotic response. They found that in hybrids with low heterotic response the nuclear DNA content exceeded the expected DNA amount, whereas in hybrids with high heterotic response the nuclear DNA content was not significantly different from that expected. Poggio *et al.* (1997) reported that cytoplasm of teosinte (*Z. mays* ssp. *mexicana*) promotes an increase in total nuclear DNA content through an increase in highly repetitive DNA in the zone of knobs in alloplasmic lines of maize.

There are many cases in which an interesting relationship between DNA amount and geographical distribution has been described (Grime and Mowforth, 1982; Bennett, 1987; Price, 1988*a*; Poggio and Naranjo, 1990). Significant

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TABLE 1. *Taxonomical classification of genus* Zea *and ranges in DNA content*

Taxa*	2*n*	Range of DNA content 2C (pg)	% of variation	Ref.
Section *Zea*				
Zea mays L.				
Zea mays ssp. *mays* Iltis & Doebley	20	4·92–6·87	40	1–8
Zea mays ssp. *mexicana* (Schrader) Iltis & Dobley	20	5·26–7·09	35	1, 8
Zea mays ssp. *parviglumis* Iltis & Doebley	20	5·59–6·19	11	1, 9
Section *Luxuriantes* Doebley & Iltis				
Zea luxurians (Durieu & Ascherson) Bird.	20	8·83–9·23	4·5	1, 8
Zea diploperennis Iltis, Doebley & Guzman	20	5·28–6·36	20·5	1, 8
Zea perennis (Hitch.) Reeves & Mangelsdorf	40	10·56–11·36	8	1, 8

Data from: 1, Laurie and Bennett (1985); 2, Rayburn *et al.* (1985); 3, Price (1988*a*, *b*); 4, Rayburn *et al.* (1989); 5, Porter and Rayburn (1990); 6, Rayburn (1990); 7, Rayburn and Auger (1990*a*, *b*); 8, Tito, Poggio and Naranjo (1991); 9, Guillin, Poggio and Naranjo (1992).

* Taxonomic nomenclature according to Iltis and Doebley (1980).

correlations between intraspecific variation in DNA amount and both latitude and altitude of cultivation have been noted for crop and non-crop species (Bennett, 1987). All these reports suggest that in higher plants genome size could be predictable, adaptive and of evolutionary significance. *Zea* is a good model to test several correlations among genome size and nuclear and environmental parameters because it shows both intra- and interspecific variation in DNA amount. Moreover, maize and related species grow in very different ecological environments. The taxonomic classification of the genus and ranges in DNA content reported for each taxa are shown in Table 1.

As mentioned previously, variation in the number and size of heterochromatic bands contributes to the differences in DNA content in maize and other species of the genus (Laurie and Bennett, 1985; Rayburn *et al.*, 1985; Tito, Poggio and Naranjo, 1991). However, repetitive sequences dispersed throughout the genome also contribute to variation in genome size (Flavell, 1982, 1986).

It is interesting to note that differences in DNA content do not interfere with pairing of chromosomes, as heteromorphic bivalents are formed which segregate normally in hybrids between *Zea* taxa that differ by about 50% in their DNA content per basic genome (e.g. *Zea luxurians* × *Z. diploperennis*; *Z. luxurians* × *Z. mays* ssp. *parviglumis*) (Poggio *et al.*, unpubl. res.). Several authors have reported the existence of relationships between genome size, heterochromatin (number of bands and number of knobs) and the

TABLE 2. *Relationship between genome size, heterochromatin, B chromosomes and environmental parameters*

Relationship	Correlation	Material	Authors
DNA content—latitude	Negative	22 North American lines	Rayburn *et al.* (1985)
DNA content—latitude	Negative	11 United States and Mexico stocks	Laurie and Bennett (1985)
DNA content—altitude	Negative	8 Mexican populations	Rayburn and Auger (1990*a*)
DNA content—altitude	Negative	12 New Mexico populations	Rayburn (1990)
DNA content—altitude	Positive	12 Arizona populations	Rayburn and Auger (1990*b*)
DNA content—VG	Positive	7 Lines and varieties	Tito *et al.* (1991)
DNA content—GDD	Positive	23 Southwestern US Indian pop.	Bullock and Rayburn (1991)
DNA content—CT and FT	Positive	8 Selected pop. Nebraska Univ.	McMurphy and Rayburn (1991)
DNA content—C-bands	Positive	22 North American lines	Rayburn *et al.* (1985)
DNA content—C-bands	Positive	11 United States and Mexico stocks	Laurie and Bennett (1985)
DNA content—C-bands	Positive	12 Arizona populations	Porter and Rayburn (1990)
DNA content—C-bands	Positive	7 Lines and varieties	Tito *et al.* (1991)
DNA content—Bs	No correl.	12 Arizona populations	Porter and Rayburn (1990)
Knobs—altitude	Negative	15 Western Guatemala populations	Mangelsdorf and Cameron (1942)
Knobs—altitude	Negative	Latin america populations	Longley and Kato-Y (1965)
Knobs—altitude	Negative	21 Mexican populations	Bennett (1976)
Knobs—altitude	Negative	300 Mesoamerican populations	Bretting and Goodman (1989)
Knobs—latitude	Negative	425 Italians populations	Bianchi *et al.* (1963)
Knobs—latitude	Negative	21 Mexican populations	Bennett (1976)
Knobs—Bs	Negative	33 North American populations	Longley (1938)
Knobs—Bs	Negative	425 Italians populations	Bianchi *et al.* (1963)
C-bands—latitude	Negative	22 North American lines	Rayburn *et al.* (1985)
C-bands—latitude	Negative	11 United States and Mexico stocks	Laurie and Bennett (1985)
C-bands—altitude	Positive	12 Arizona populations	Porter and Rayburn (1990)
C-bands—VP	Positive	7 Lines and varieties	Tito *et al.* (1991)
C-bands—CT and FT	Positive	8 Selected pop. Nebraska Univ.	McMurphy and Rayburn (1992)
C-bands—Bs	No correl.	12 Arizona populations	Porter and Rayburn (1990)
Bs—altitude	Negative	300 Mesoamerican populations	Bretting and Goodman (1989)
Bs—altitude	No correl.	12 Arizona populations	Porter and Rayburn (1990)

Bs, B chromosomes; VP, vegetative period; GDD, growing degree days unit (effective growing season); CT, cold tolerance; FT, freeze tolerance; pop, populations.

occurrence of supernumerary chromosomes in maize and environmental parameters such as latitude and altitude (see Table 2). Generally, in different places, at different altitudes and latitudes, different relationships are found (positive or negative correlations), but in most cases the results indicate the existence of adaptive clines.

The addition of either heterochromatin or supernumerary chromosomes (Bs) to one individual results in an increase in genome size. In fact, Ayonoadu and Rees (1971) studied genome size in individuals with zero or eight B chromosomes in the line Black Mexican sweet corn and calculated that each B increased the DNA content by about 5%.

Could the same rule be applied to populations with B chromosomes and polymorphism for heterochromatic bands? Is the relationship between genome size and environmental parameters similar whether the increase in DNA content is produced by addition of knobs or by addition of B chromosomes?

To answer these questions, the genome size of 21 native populations of maize from northern Argentina cultivated at different altitudes (80–3600 m) was analysed. These populations do not interbreed with one another and are polymorphic (intrapopulational variation) and polytypic (interpopulational variation) for the number of knobs. Moreover, they have different frequencies of B chromosomes.

MATERIALS AND METHODS

Plant materials

The materials were collected by the authors from original indigenous populations and were deposited in the seedbanks of the Vavilov Laboratory (Facultad de Agronomía, Universidad de Buenos Aires) and of the Instituto Fitotécnico de Santa Catalina (Universidad de la Plata). The original populations are isolated, with no input from other populations and are maintained by open pollination. Samples were obtained from a mixture of all individuals available in each population. These populations are cultivated in Catamarca, Jujuy, Tucuman, Salta and Formosa provinces. The names of the races, and the altitudes of the provenance of the populations are shown in Table 3.

Chromosome determinations

Seeds were germinated and identified in Petri dishes. Primary root tips, 0·5–1 cm in length, of each identified germinating seedling, were fixed in 3:1 (ethanol-acetic acid), and stored at 5 °C until use. Later, lateral root tips of each identified seedling were pre-treated with 0·002 M 8-hydroxyquinoline for 3 h and fixed in 3:1. Finally, the DNA content was measured in primary roots, while the number of B chromosomes was scored in lateral root tips. They were hydrolysed in 5 N HCl at 20 °C for 12 min, rinsed three times in distilled water and stained with 2% haematoxilin and ferric citrate as mordant (Núñez, 1968). In this way, the number of Bs in each individual whose DNA content was measured could be known.

Determination of DNA content

DNA content was measured in 20 telophase nuclei (2C) of the primary root tips of germinating seedlings; three to 18 individuals per population were studied. Maize flint opaque-2 line was used as a standard to calculate genome size in picograms; its genome size was 2C = 6·658 pg, and was calibrated according to Bennett and Smith (1976) using

TABLE 3. *Races, mean DNA content, range of DAPI bands, and mean number of Bs in populations cultivated at different altitudes*

Race	Population	Altitude (m)	Mean A-DNA content $\bar{x} \pm$ s.e. (pg) (no. ind.)*	Range of bands	Mean no. of Bs per plant (no. ind.)*
Altiplano	VAV 6473	3620	6·488 ± 0·196 (6)	2–3	0·000 (43)
Altiplano	VAV 6474	3520	6·514 ± 0·053 (7)	2–3	0·000 (40)
Harinoso	VAV 6475	3240	6·491 ± 0·106 (8)	2–4	1·100 (50)
Altiplano	VAV 6167	3000	5·008 ± 0·250 (4)	2–3	1·029 (34)
Capia rosado	VAV 6162	2900	5·74 ± 0·226 (3)	—	0·730 (37)
Blanco	VAV 6485	2670	5·800 ± 0·094 (6)	1–3	1·795 (44)
Capia blanco	VAV 6418	2600	5·632 ± 0·052 (5)	—	1·020 (48)
Pisingallo	VAV 6416	2600	—	—	0·927 (41)
Amarillo grande	VAV 6480	2420	6·105 ± 0·102 (8)	—	1·596 (52)
Blanco	VAV 6479	2180	6·38 ± 0·076 (3)	—	2·615 (52)
Amarillo chico	VAV 6484	2010	6·351 ± 0·109 (7)	2–4	0·204 (49)
Amarillo chico	VAV 6451	2000	5·665 ± 0·219 (6)	5–12	0·900 (110)
Chiriguano	VAV 6218	2000	—	—	0·276 (29)
Amarillo chico	VAV 6476	1690	6·282 ± 0·176 (6)	3–6	0·255 (51)
Pisingallo	VAV 6313	1600	6·149 ± 0·101 (18)	6–12	0·685 (184)
Blanco y ocho rayas	VAV 6483	1250	6·601 ± 0·124 (4)	7–12	0·111 (36)
Orgullo cuarentón	VAV 6482	910	6·148 ± 0·082 (6)	—	0·122 (49)
Blanco y ocho rayas	VAV 6481	750	6·757 ± 0·052 (6)	6–14	0·255 (55)
Colorado	VAV 6169	80	—	—	0·323 (34)
Colorado	VAV 6223	80	—	—	0·051 (39)
Pichingá	VAV 6170	80	6·172 ± 0·101 (4)	—	0·023 (43)

* Data from Rosato *et al.*, 1998.

Allium cepa 'Ailsa Craig' (2C = 33·55 pg) (Rosato, Naranjo and Poggio, 1997*b*). The root tips of the maize standard line were fixed simultaneously as described above.

The staining method was performed as described in Tito *et al.* (1991). After fixation, roots were rinsed for 30 min in distilled water. Hydrolysis was carried out in 5 N HCl at 20 °C for 30 min in a waterbath. Roots were then washed three times in distilled water for 15 min, and stained for 2 h in Schiff's reagent at pH 2·2 (Teoh and Rees, 1976). Root tips were then rinsed three times in SO_2 water for 10 min each, kept in distilled water, and squashed in 45% acetic acid. Coverslips were removed after freezing with CO_2, and the preparations dehydrated in absolute alcohol, mounted in Euparal and maintained in the dark until measurements were made. The amount of Feulgen staining per nucleus, expressed in arbitrary units, was measured at a wavelength of 570 nm using the scanning method with a Zeiss Universal Microspectrophotometer (UMSP 30), and finally expressed in picograms by reference to the standard line.

Fluorescent chromosome banding

The pre-treated and fixed primary root tips were treated with enzymes: root samples were rinsed in 0·01 M citric acid—sodium citrate buffer for 30 minutes, and then softened in 2% cellulose (1·6% Calbiochem + 0·4% Ozonuka-R10) plus 20% pectinase solution at 37 °C for 90 min. Afterwards, root tips were washed in the same buffer and squashed in 45% acetic acid. Coverslips were removed after freezing with CO_2 and the slides were dried and stored overnight or longer before staining.

DAPI staining. Slides were washed in McIlvaine buffer (citric acid-Na_2HPO_4 buffer pH 6·9–7·0), and then stained with DAPI (1 μg ml^{-1}). Slides were incubated in a moist box at 20 °C, in the dark, for 25 min. After staining, the preparations were briefly washed with distilled water, McIlvaine buffer and then distilled water again. Slides were mounted in McIlvaine buffer and sealed with rubber solution.

A Zeiss Axiophot microscope with a mercury lamp (HBO 50 W) was used to view chromosome fluorescence. Slides were illuminated with Zeiss filter set 1 (excitation 360 nm, emission 460 nm) for DAPI and immediately photographed on Kodak T-Max 400 black and white negative film.

Statistical analysis

Simple correlations among A-DNA content and altitude, A-DNA content and mean number of bands, A-DNA content and mean number of Bs, mean number of Bs and altitude, mean number of Bs and mean number of bands, and mean number of bands and altitude were calculated using the Statgraphics plus (7·1) program. Two-way analysis of variance was used to evaluate differences in DNA content between populations and doses of Bs. Differences within each population were tested by one-way ANOVA and comparisons between means of DNA content were made using Scheffe's method. The frequency of DAPI bands between individuals with and without Bs in the population VAV 6475 was analysed with a χ^2 test.

RESULTS AND DISCUSSION

A-DNA content in populations cultivated at different altitudes

The DNA content of the regular complement (A-DNA) of 17 populations (107 individuals) was determined (Table 3). The range of variation was 36% (5·008–6·756 pg) among the studied populations (Rosato *et al.*, 1998). Measurements were performed in plants without Bs ($2n = 20$) with the aim of studying the variation in DNA content of A chromosomes (A-DNA) independently from the variation caused by Bs. The range in variation found among these populations is similar to that found in populations from the USA and Mexico (Laurie and Bennett, 1985; Rayburn *et al.*, 1985; Porter and Rayburn, 1990; Rayburn, 1990; Rayburn and Auger, 1990*a*, *b*).

Several authors have analysed the total DNA content of maize populations adapted to various altitudes. These results are summarized in Table 2. Although there are some discrepancies, all these results lead to the conclusion that low genome size is characteristic of maize at elevations above about 2000 m. In 15 of the populations discussed in the present work, a significant negative correlation with altitude of cultivation was demonstrated (Table 4). The correlation between A-DNA and altitude only involved populations with numerical polymorphism for B chromosomes. When the two populations with high A-DNA content (Altiplano VAV 6473 with 2C = 6·48 ± 0·129 pg and Altiplano VAV 6474 with 2C = 6·51 ± 0·053 pg), which did not show B-chromosome polymorphism, were included in the analysis, the correlation between A-DNA and altitude was not significant. This could be due to the fact that these populations are cultivated in marginal areas in the Andean region of the Jujuy Province under extreme environmental conditions for this crop. Moreover, they do not contain B chromosomes, which are very frequent at high altitudes and they also have few heterochromatic bands in relation to their high A-DNA content. This lack of correlation between A-DNA and altitude in these two populations is probably related to their cold and freezing tolerance. McMurphy and Rayburn (1991, 1992) analysed genome size variation in maize populations selected for cold and freezing tolerance. They found that populations selected for cold tolerance and with a significant degree of freezing tolerance had a larger genome size and a higher number of C bands compared to unselected populations; Altiplano VAV 6473 and VAV 6474 populations (probably cold and freezing tolerant)

TABLE 4. *Correlation between nuclear DNA and environmental parameters*

Correlation	*r*	*P*
A-DNA content—altitude	−0·3134	0·0022
Number of bands—altitude	−0·726	< 0·0001
Mean no. of Bs—altitude	0·6355	0·0035
Number of bands—mean no. of Bs	−0·54487	< 0·00001
A-DNA content—mean no. of Bs	−0·3392	0·0003

could show a similar phenomenon. In the case of these two populations, however, the C band number did not fluctuate in concert with the DNA amount.

The negative correlation between A-DNA and altitude found in Argentine populations is in agreement with the majority of previous results (Table 2). However, it is interesting to point out that we are exclusively studying DNA content of A-chromosomes (individuals without Bs).

Relationship between A-DNA content, heterochromatin, B-chromosomes and altitude of cultivation

Heterochromatic blocks in maize are visualized as knobs at the pachytene stage of meiosis (McClintock, 1929). They comprise a highly repeated DNA sequence of 185 bp (Peacock *et al.*, 1981). Ward (1980) determined that the knobs in pachytene correspond to the C bands observed at mitosis. It is possible to learn the composition of the heterochromatin using G-C and A-T specific fluorochromes such as CMA and DAPI, respectively (Sumner, 1990). In this way, two classes of heterochromatin (G-C rich DNA—CMA + bands and A-T rich DNA—DAPI + bands) could be differentiated. The sum of both types of fluorescent bands coincides with C bands (Schweizer, 1976). Variation in the amount of heterochromatin (C bands) is an important cause of differences in DNA content of *Zea* taxa (Table 2). Several authors have analysed the relationship between C bands, knobs and environmental parameters and found that there are a greater number of C bands or knobs in samples from lower altitudes and latitudes compared with those from higher altitudes and latitudes (Table 2).

To examine the relationship between heterochromatin and altitude in Argentine populations of maize growing at different altitudes (750–3600 m), fluorescent chromosome banding (DAPI) was analysed in 11 populations differing in their A-DNA content (Table 3). Only DAPI bands were considered because CMA bands were always present in the proximal zone of the NOR of chromosome 6 in all the individuals studied. The number of DAPI bands exhibited both intra- and interpopulation variation (Fig. 1). In populations from higher altitudes (over 2000 m) there was a narrow range of intra- and interpopulation variation (one–four bands) while at lower altitudes the range was wider (three–14 bands). The number of bands showed a significant negative correlation with altitude of cultivation (Tables 3 and 4). These results are in agreement with most previous reports (Table 2). Tito *et al.* (1991) determined that the percentage of heterochromatin is positively correlated with the vegetative period. Price (1988*a*) and Rayburn *et al.* (1985) considered that the decrease in the number of C bands (knobs) could be an adaptation to a shorter growing season and the result of artificial selection by man. Therefore, the low mean number of DAPI bands found at high altitudes (Table 3) could be related to the length of the growing season.

No statistical differences were found between A-DNA content and number of bands, because in different populations individuals with high A-DNA content could possess high or low number of bands. This is not surprising since sequences not arranged in tandem could be contributing to the A-DNA content variation. In fact, several authors found variation in the number of copies of different classes of sequences including middle repetitive DNA, highly repetitive DNA and microsatellites (Hake and Walbot, 1980; Flavell, 1986; Rivin, Cullis and Walbot, 1986; Bennetzen *et al.*, 1994; Chin *et al.*, 1996). Moreover, there are examples of variation in sequences undetected by banding techniques in other species of plants such as flax and sunflower (Cavallini *et al.*, 1986; Cullis and Cleary, 1986; Natali *et al.*, 1993).

B-chromosomes are another source of DNA content variation. Bs are widely distributed in maize and are found in several lines, including commercial varieties, and at high frequencies in native populations (Longley, 1927, 1938; Randolph, 1928; Avdulow, 1933; Carlson, 1978; McClintock, Kato and Blumenschein, 1981; Rosato *et al.*, 1998). The Bs have non-Mendelian inheritance showing accumulation mechanisms (Roman, 1947, 1948; Carlson, 1969; Carlson and Chou, 1981; Carlson and Roseman, 1992; Rosato *et al.*, 1996).

Rosato *et al.* (1998) studied 21 native populations (1120 individuals) of maize from northern Argentina cultivated at different altitudes (80–3620 m). Nineteen of the populations analysed showed numerical polymorphism for B chromosomes. The mean number of Bs per plant varied from 0 to 2·615 (Table 3). The number of Bs per plant varied from zero to eight, the predominant doses being zero, one, two and three. Notably, there was a positive and statistically significant correlation between number of Bs and altitude of cultivation (Tables 3 and 4), which was not shown in populations without Bs (VAV 6474 and 6473), which grow at 3520 and 3620 m, respectively. These populations occur in a marginal area for the common distribution of maize, in extreme environmental conditions. In *Crepis capillaris*, for example, Bs are only tolerated in populations that are not under severe selective stress (Parker *et al.*, 1991). Our results also indicate that maize B chromosomes are tolerated in populations without severe selective pressure.

The mean number of Bs shows a significant negative correlation with the A-DNA content in 17 of the populations studied. In 11 of these populations, the number of DAPI bands was analysed and a significant negative correlation between Bs and number of bands was found (Tables 3 and 4). These facts are also in agreement with the negative association between the number of knobs and the occurrence of B-chromosomes in North American indian and Italian populations of maize found by Longley (1938) and Bianchi, Ghatnekar and Ghidoni (1963), respectively. These results suggest that Bs are tolerated at high frequencies in those populations with a low A-DNA content and low number of bands, and this could indicate a maximum limit to the mass of nuclear DNA or nucleotype and that Bs are tolerated so long as this maximum limit is not exceeded. Finally, Rosato *et al.* (1998) discuss the adaptative significance of the clinal variation of A-DNA content and number of bands and the consequent inverse correlation of mean number of Bs over an altitudinal gradient.

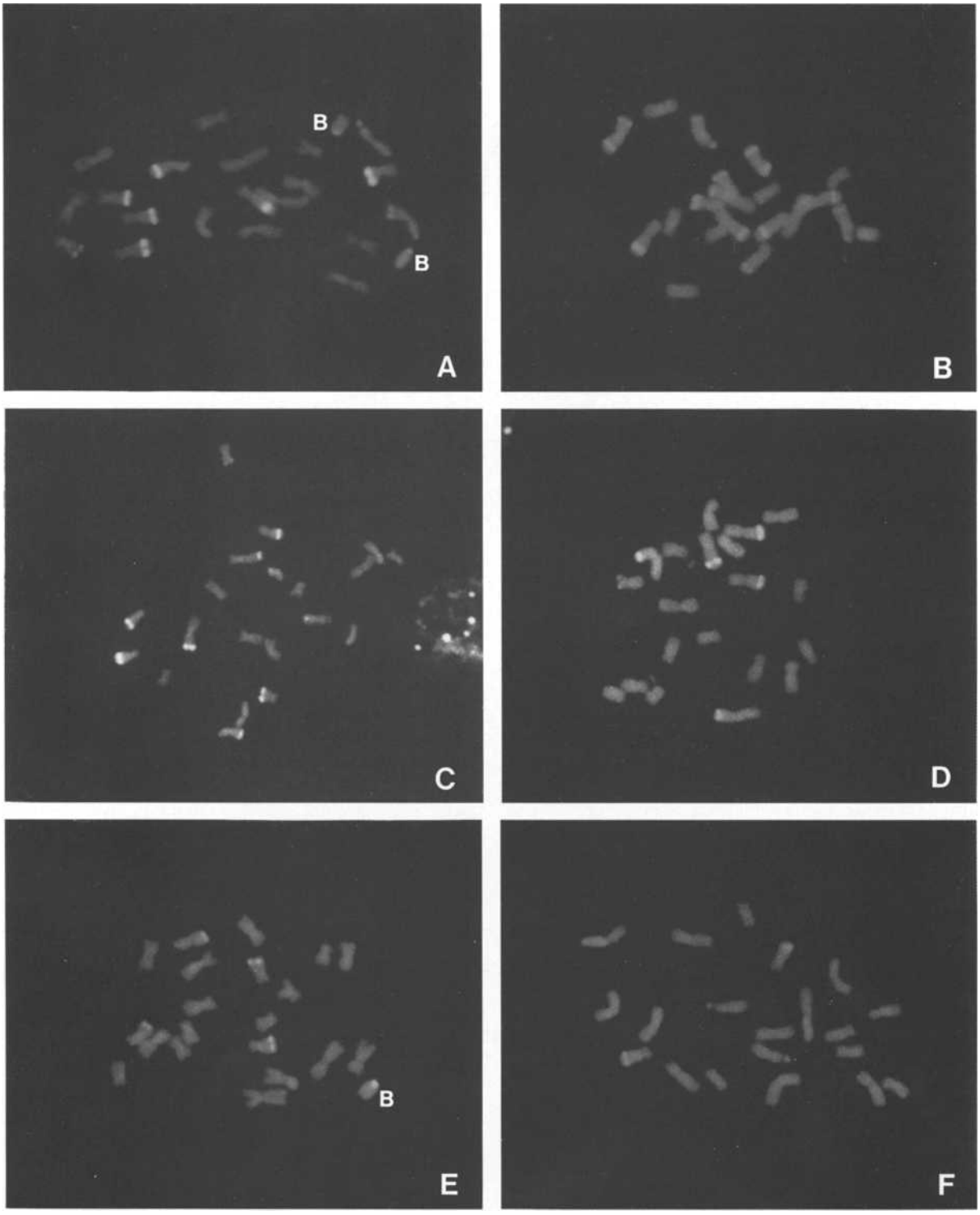

FIG. 1. Fluorescent chromosome banding (DAPI) in mitotic metaphase. A and B, VAV 6481. A, $2n = 20+2B$, 8 bands in A-chromosomes (As). B, $2n = 20$, 15 bands. C, VAV 6483, $2n = 20$, 8 bands. D, VAV 6451, $2n = 20$, 5 bands. E, VAV 6475, $2n = 20+1B$, 4 bands. F, VAV 6485, $2n = 20$, 2 bands.

TABLE 5. *DNA content in individuals with different doses of Bs in four populations*

Population (Race)	Mean DNA content with different doses of Bs (mean ± s.e.) (pg) (no. ind)			
	0B	1B	2B	3B
VAV 6485 (Blanco)	5·800 ± 0·094 (6)	6·310 ± 0·072 (3)	6·392 ± 0·062 (4)	6·599 ± 0·146 (4)
VAV 6480 (Am. grande)	6·105 ± 0·102 (8)	6·085 ± 0·083 (4)	6·285 ± 0·097 (4)	6·423 ± 0·016 (3)
VAV 6479 (Blanco)	6·380 ± 0·076 (3)	6·074 ± 0·050 (6)	6·375 ± 0·070 (4)	6·622 ± 0·068 (4)
VAV 6475 (Harinoso)	6·491 ± 0·106 (6)	5·786 ± 0·145 (3)	6·377 ± 0·053 (4)	6·592 ± 0·081 (4)

Relationship between number of bands and doses of Bs with total DNA content

We investigated whether an increase in the number of Bs is related to a concerted increase in the total DNA amount in native races of maize. In Table 5 the total DNA content of individuals of four native populations with different doses of B are summarized. Two-way analysis of variance showed that only the B-chromosome dose × population interaction was significant ($F = 4·3536$, $P = 0·0003$), indicating that variation in DNA content with the dose of Bs is population-dependent. The results showed that in population VAV 6485, with the lowest A-DNA value (5·8 pg in individuals without Bs), the DNA content of individuals with Bs was significantly higher than in individuals without Bs (Table 5). In population VAV 6480, individuals with different doses of Bs did not show any significant differences in DNA content (Table 5). In the other two populations, VAV 6479 and VAV 6475 with the highest A-DNA values (6·409 and 6·448 pg, respectively), the DNA content in individuals without Bs was equal (VAV 6479) or even significantly higher (VAV 6475) than in individuals with one B (6·074 and 5·786 pg, respectively). In the latter populations, the increase in DNA content with dose of Bs becomes more evident as the number of Bs increases from one B (Table 5). These results support the aforementioned hypothesis that there is a maximum nucleotype limit, because when the A-DNA content is high there would be no detectable increase in total DNA amount in individuals with low doses of Bs.

In view of these results, individuals with Bs do not always have a higher DNA content than individuals without Bs. For this reason, it is not possible to predict the genome size of individuals with different doses of Bs, considering only a 5% increase in DNA content in native populations per B as determined by Ayonoadu and Rees (1971) in an inbred line. There may be variation in the A-DNA content within these populations masking the increase due to the presence of Bs.

To determine the cause of this masking effect, we analysed the fluorescent chromosome banding pattern in individuals with different doses of Bs in two populations (VAV 6475 and VAV 6485). The individuals from VAV 6475 showed two, three or four DAPI bands which were always on chromosomes 6 and 9 (Fig. 1E). All the individuals were homozygous for the presence of the DAPI band in the chromosome pair 9. In contrast, the DAPI band in chromosome pair 6 was polymorphic, i.e. individuals were homozygous (presence or absence) and heterozygous for the DAPI band. The mean number of DAPI bands in chromosome pair 6 in individuals with Bs (0·42 in 29 individuals) was lower than that in individuals without Bs (1·00 in 18 individuals) (Table 6), and this association was significant (Yates $\chi^2 = 5·76$, $P = 0·0164$), showing that in population VAV 6475 the presence of the band in chromosome pair 6 is not independent of the presence or absence of B-chromosomes.

TABLE 6. *Mean number of DAPI bands in chromosome pair 6 in two populations*

Population	Mean number of DAPI band in the chromosome pair 6 (no. ind)				
	0B	1B	2B	3B	Indiv. with Bs
VAV 6475	1·00 (18)	0·50 (11)	0·36 (11)	0·40 (7)	0·42 (29)
VAV 6485	0·17 (12)	0·00 (7)	0·50 (4)	0·17 (6)	0·18 (17)

Individuals from VAV 6485 showed DAPI bands in the same chromosome pairs (6 and 9). Two out of 29 plants were heterozygous for the presence of the band in chromosome pair 9 (Fig. 1F), but there was more polymorphism for the band on chromosome 6. In Table 6, only the band of chromosome pair 6 was considered. The mean number of DAPI bands was similar in individuals with and without Bs (0·18 in 18 individuals and 0·17 in 12 individuals, respectively). Therefore, in population VAV 6485, the distribution of the number of DAPI bands is similar between individuals with and without B-chromosomes. The masking effect, observed in populations VAV 6479, VAV 6480 and VAV 6475, could be associated with a low mean number of DAPI bands in individuals with Bs. Other dispersed repetitive DNA sequences could also be involved as was suggested by Porter and Rayburn (1990). On the contrary, in population VAV 6485, which did not show any masking effect, there was no variation in mean number of bands between individuals with and without Bs.

CONCLUSIONS

(1) A large range of variation in DNA content (36%) among populations was found. This variation corresponds to differences in DNA content of the regular A-chromosome complement (A-DNA) irrespective of B-chromosomes; (2) the clinal variation of A-DNA content and number of heterochromatic bands, and the consequent inverse cor-

relation of mean number of Bs over an altitudinal gradient, could have an adaptative significance; (3) the negative correlation between A-DNA content and mean number of Bs suggests that Bs are better tolerated in populations with a lower A-DNA content; (4) B-chromosomes are a source of intraspecific variation in DNA content; however, this variation might not be detected in maize populations polymorphic for heterochromatin. Therefore, a negative association between Bs and heterochromatic bands would exist to maintain an optimum nucleotype.

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