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Cytogenetic studies in the genus *Zea*

3. DNA content and heterochromatin in species and hybrids

C.M. Tito [1,2], L. Poggio [1,3] and C.A. Naranjo [1]

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET),
[1] Argentina Instituto Fitotécnico de Santa Catalina, 1836 Llavallol, Buenos Aires, Argentina
[2] Fellowship of the Com. Inv. Cient. Bs. As. (CIC)
[3] Facultad de Cs. Ex. y Nat., U.B.A.

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Summary. The nuclear DNA amount and the heterochromatin content in species and hybrids of *Zea* were analyzed in telophase nuclei (2C) of the root apex of germinating seeds. The results revealed significant differences among taxa and also among lines and races of maize. The hybrids between *Z. mays* ssp. *mays* × *Z. mays* ssp. *mexicana* (2n = 20), *Z. diploperennis* × *Z. perennis* (2n = 30), and *Z. diploperennis* × *Z.perennis* (2n = 40) showed DNA content intermediate between that of the parents. The number of chromosomal C-bands and the proportion of the genome comprising C-band heterochromatin were positively related to genome size. In the different lines and races of maize studied, there was a positive correlation between genome size and the interval from germination to flowering. Octoploid *Z. perennis* (2n = 40) showed the smallest DNA content per basic genome and the smallest heterochromatic blocks, suggesting that the DNA lost by this species consisted mainly of repetitive sequences. Considering that the extant species of *Zea* are tetraploid (2n = 20) and octoploid (2n = 40) and that the ancestral diploids are extinct, any consideration of the direction (increase or decrease) of the DNA change would be entirely speculative. The extant species could be the product of natural and artificial selection acting on different genotypic and nucleotypical constitutions at the diploid and/or tetraploid levels.

Key words: *Zea* species and hybrids – Cytogenetics – DNA content – Heterochromatin – C-banding

Introduction

A large intra- and interspecific variation in DNA content per genome has been found in higher plants. The lack of correlation between organismic complexity and DNA content, the variation in DNA amount among closely related species, and the fact that only a fraction of the total DNA is necessary for protein coding has been called the C-value paradox (Thomas 1971). In general, changes in DNA content involve gain or loss of repetitive DNA sequences (Flavell 1986; Price 1988a, b). These changes modify the nucleotype, which Bennett (1972, 1987) defined as the effect of nuclear DNA quantity on the phenotype, independent of its encoded informational function. Thus, the nucleotype is determined by the total nuclear DNA content (genetic and nongenetic), and it influences several cellular and developmental parameters such as chromosome size, nuclear volume, cell volume, mitotic cycle time, duration of meiosis, minimum generation time, etc. (Bennett 1987; Grant 1987; Price 1988a, b).

Many reports point out that in higher plants the effects of total DNA content variation are predictable, adaptative, and of evolutionary significance (Price 1976, 1988a, b; Bennett 1972, 1987).

The genus *Zea* is an interesting example of intra- and interspecific variation of DNA amount. Brown (1949) reported that the number of knobs in maize (*Z. mays* ssp. *mays*) decreases with increasing latitude in North America; this also occurs with increasing latitude in Mexico (Bennett 1976). On the other hand, Rayburn et al. (1985) determined the number of mitotic C-bands, the proportion of genomes comprising C-band heterochromatin, and the genome size (4C-DNA content) for 22 North American inbred and open-pollinated lines of maize. Later authors found a significant negative correlation between DNA content and latitude and a significant positive correlation between genome size and the amount of heterochromatin. Rayburn et al. (1989) studied the DNA content variation among corn lines using flow cytometry,

and found that the results were in agreement with the amount of variation observed in their previous paper (Rayburn et al. 1985) using microdensitometry.

Laurie and Bennett (1985) showed that DNA content varies significantly in the genus *Zea* and within *Z. mays* ssp. *mays*. They found that in *Z. mays* ssp. *mexicana*, *Z. mays* ssp. *parviglumis*, and *Z. diploperennis* the DNA contents were within the range recorded for maize, while in *Z. luxurians* it was about 50% higher. The papers by Laurie and Bennett (1985) and Rayburn et al. (1985) provide convincing evidence that variation of DNA content in *Zea* is largely caused by differences in the amount of heterochromatin and that it is also of adaptive significance.

In the present investigation, the 2C DNA content of several species and subspecies of *Zea* and of intra- and interspecific hybrids and lines differing in their vegetative period is examined. The goal of this work is to supplement information on the extent of variation of the genome size in the genus and to analyze the correlation between total nuclear DNA content, number of C-bands, proportion of heterochromatin, and the vegetative period (interval from germination to flowering) in the studied taxa. Moreover, the determination of the genome size and the C-banding pattern throughout the genus would be an important contribution to previous studies, which proposed x = 5 as basic chromosome number and a new genomic constitution for the different species of *Zea* (Molina and Naranjo 1987; Naranjo et al. 1990; Poggio et al. 1990a).

Materials and methods

Plant materials

The material was cultivated at the "Instituto Fitotécnico de Santa Catalina" (IFSC), Llavallol.

Z. mays ssp. *mays*. "C-tester inbred line" introduced by Horowitz from the United States; "Accession 9063": commercial hybrid; "SC6 inbred line": single hybrid; "Ever Green": population selected at the IFSC; "Colorado Klein": population selected at the IFSC; "Z inbred line": introduced from the United States in 1934, designated "Multiple Dominante, Dr. Randolph 1877"; "E line": raised by L. B. Mazoti, using Z line as male recurrent parent during 20 backcrosses by *Z. mays* ssp. *mexicana* (Florida variety, Huixtla, Mexico).

Z. mays ssp. *mexicana* (CIMMYT K69-5), cultivated since 1980. *Z. luxurians:* University of Guadalajara (CIMMYT). *Z. diploperennis:* Mexico, Jalisco, Sierra de Manathan occidental, R. Guzmán & M. A. de Guzmán (1120), cultivated since 1980. *Z. perennis:* Mexico, Jalisco, the city of Guzmán, Dra. Prywer, cultivated at the IFSC since 1962.

The classification of the genus *Zea* used in this paper is as given by Doebley and Iltis (1980) and Iltis and Doebley (1980).

Determination of DNA content

DNA content was measured in telophase nuclei (2C) of the root apex of germinating seeds. Seeds were placed in petri dishes on wet filter paper. Roots of 0.5–1 cm in length were fixed in 3:1 (absolute ethanol:acetic acid) for 1–4 days. After fixation, the roots were rinsed for 30 min in distilled water. Hydrolysis was carried out with 5 *N* HCl at 20 °C. Different durations of hydrolysis were investigated and the optimum period was found to be 30 min. The roots were then washed three times in distilled water for 10 or 15 min, and stained for 120 min in Schiff's reagent at pH 2.2 (Teoth and Rees 1976). The material was then rinsed three times in SO_2 water for 10 min each, kept in distilled water (5–15 min), and squashed in 45% acetic acid. The coverslip was removed after freezing with CO_2 and the slide was dehydrated in absolute alcohol and then mounted in Euparal. 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).

The DNA content per basic genome, expressed in picograms, was calculated using *Allium cepa* var. Ailsa Craig as a standard (2C = 33,55 pg; Bennett and Smith 1976). The differences in DNA content were tested by an analysis of variance and comparisons between means using Scheffe's method.

C-banding

The C-banding technique using Giemsa-staining was performed according to Giraldez et al. (1979). The area of heterochromatin per interphase nucleus was obtained by measuring the area of each chromocenter C+ within each nucleus. The measurements were carried out using a Mini-Mop (Kontron) Image Analyzer, working with photomicrographs.

The sum of all chromocenter areas was made for each of 30 nuclei of similar surfaces. The relative heterochromatin area of each taxon was compared to that of *Z. luxurians*, which is the tetraploid species (2n = 20) with the highest 2C DNA and heterochromatin content.

Results

The DNA content expressed in picograms, the number of C-bands at mitotic metaphase, the proportion of heterochromatin, and the vegetative period are indicated in Table 1. Four experiments were performed. In each experiment, two to four replicates were measured for each taxon, and the measurements were pooled for comparisons, as they did not show significant differences.

Experiment 1

Comparison among DNA content in *Z. diploperennis*, *Z. perennis*, and *Z. diploperennis* × *Z. perennis* (2n = 30 and 2n = 40; Table 1).

Analysis of variance showed significant differences between taxa ($F = 203.3$; $P < 0.01$). Comparisons by Scheffe's method for *Z. diploperennis* indicated significant differences from the rest of the taxa, while *Z. perennis* showed significant differences from the hybrid *Z. diploperennis* × *Z. perennis* (2n = 30), and nonsignificant differences from *Z. diploperennis* × *Z. perennis* (2n = 40). On the other hand, the difference between both hybrids was significant.

Table 1. DNA content, proportion of heterochromatin, C-band numbers in mitotic metaphase, and vegetative period in species and hybrids

TAXA	2n	Ploidy level	Veget.[a] period (days)	No. of[b] nuclei	% hetero-chromatin	C-band[c] number	DNA content (2C) pg $X \pm SE$	DNA per basic[d] genome pg
Section Zea								
Z. mays ssp. *mays* line C-tester	20	4×	A (52)	95 (3)	16.97	4 (4)	5.86±0.05	1.46[ad]
Z. mays ssp. *mays* var. "Ever Green"	20	4×	A (65)	68 (2)	22.90	6 (6)	6.06±0.06	1.52[ad]
Z. mays ssp. *mays* 9063	20	4×	A (75)	89 (3)	–	12 (12)	6.16±0.07	1.54[a]
Z. mays ssp. *mays* var. "Colorado Klein"	20	4×	A (75)	80 (3)	25.16	12 (12)	6.19±0.06	1.54[a]
Z. mays ssp. *mays* line Z	20	4×	B (84)	155 (4)	47.41	10 (10)	6.76±0.04	1.69[b]
Z. mays ssp. *mays* line SC.6	20	4×	B (103)	90 (3)	49.70	10 (10)	6.87±0.06	1.72[bc]
Z. mays ssp. *mays* line E	20	4×	B (91)	157 (4)	50.28	10 (10)	7.09±0.04	1.77[c]
Z. mays ssp. *mexicana* K 69-5	20	4×	C (170)	141 (4)	69.10	18 (18)	6.79±0.05	1.70[bc]
Z. mays ssp. *mays* (9063) × *Z. mays* ssp *mexicana* K 69-5	20	4×		90 (4)	–	–	6.56±0.07	
Section Luxuriantes								
Z. diploperennis	20	4×	D (190)	97 (3)	58.93	20 (20)	6.36±0.06	1.59[a]
Z. perennis	40	8×	E (200)	62 (2)	39.09	– (30)	11.36±0.11	1.42[d]
Z. luxurians	20	4×	F (212)	81 (3)	100	22	8.83±0.08	2.20[e]
Z. d. × *Z. p.*	30	6×		92 (4)	–	–	8.54±0.07	–
Z. d. × *Z. p.*	40	8×		81 (4)	–	–	11.57±0.09	–

[a] Interval from germination to flowering: A = 52–75 days; B = 84–103 days; C = 170 days; D = 190 days; E = 200 days; F = 212 days
[b] Number of replicates between brackets
[c] Maximum number of C+ chromocenters at interphase between brackets
[d] Means with the same letter are not signficantly different

Experiment 2

Comparison among nuclear DNA content of *Z. mays* ssp. *mays* (9063), *Z. mays* ssp. *mexicana* (K-69-5), and their hybrid (Table 1).

The analysis of variance and Scheffe's method indicated significant differences among the taxa ($F = 83.47$; $P < 0.01$).

Experiment 3

Comparisons among lines and populations of *Z. mays* ssp. *mays* (Table 1).

The analysis of variance showed highly significant differences between them ($F = 40.69$; $P < 0.01$). Scheffe's test indicated that the differences within the group having a short vegetative period (C-tester, "Ever Green", 9063, and "Colorado Klein") were nonsignificant. On the other hand, there are significant differences among lines with longer vegetative periods (SC6, Z, E). Scheffe's test indicated that the group with shorter vegetative period (A, Table 1) had significant differences from lines with longer vegetative period (B, Table 1).

Experiment 4

Comparisons among *Z. luxurians, Z. diploperennis,* and *Z. perennis* (Table 1).

Analysis of variance and Scheffe's method showed significant differences among the taxa ($F = 81.24$; $P < 0.01$). The data from the four experiments were pooled and the DNA content per basic genome (*C*-value) was calculated. The analysis of variance indicates significant differences in the C-value among the taxa listed in Table 1 ($F = 35.78$; $P < 0.01$). Comparisons made through Scheffe's method indicated: (a) In *Z. mays* ssp. *mays,* lines with a shorter vegetative period (A) differ significantly from lines with longer cycles (B). (b) *Z. mays* ssp. *mexicana* differs significantly from maize lines with short

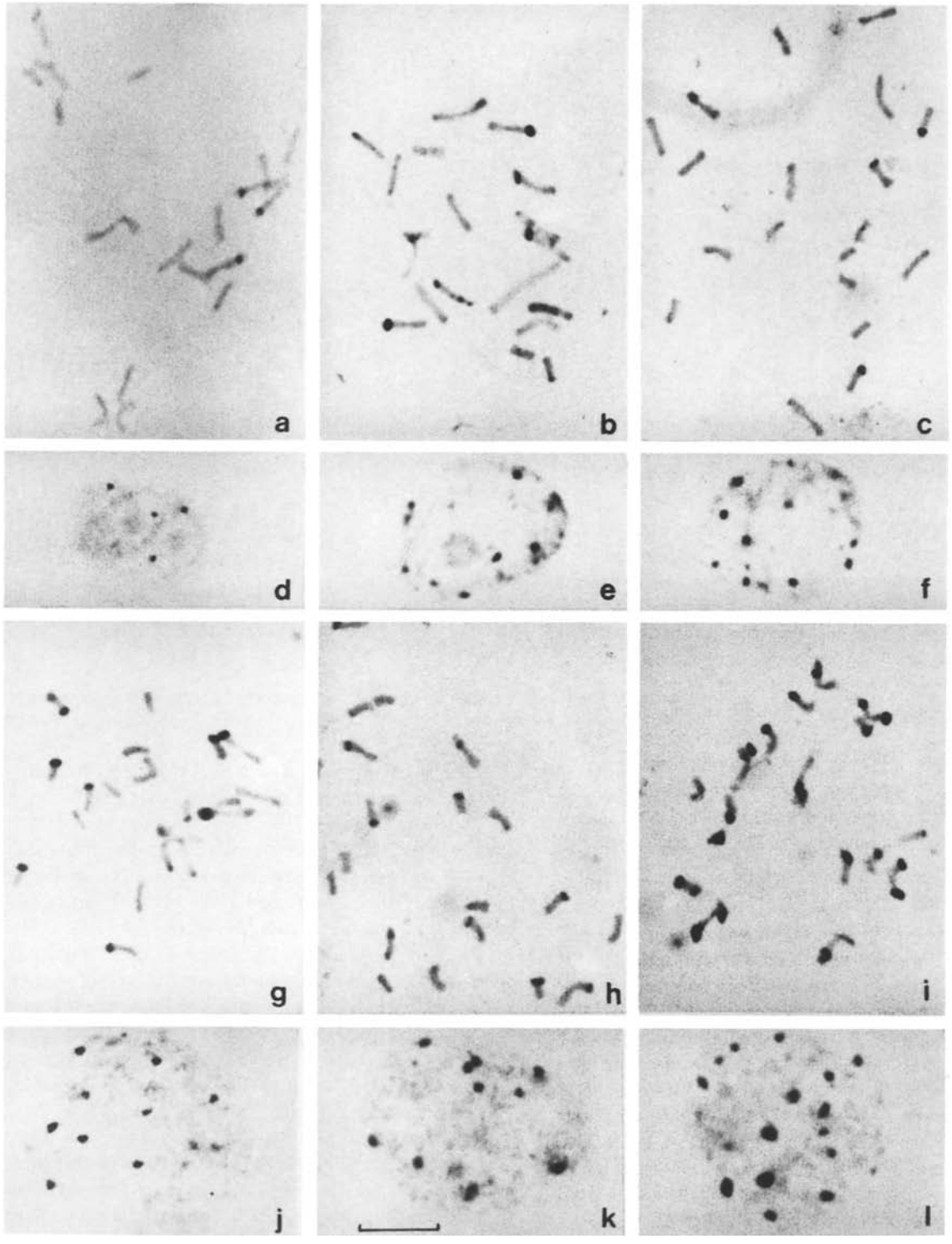

Fig. 1 a–l. C-banding. **a–c** and **g–i** = mitotic metaphase; **d–f** and **j–l** = interphase nuclei. **a–h** and **j, k** = *Z. mays* ssp. *mays* (**a** and **d** = C-tester; **b** and **e** = var. "Ever Green"; **c** and **f** = var. "Colorado Klein"; **g** and **j** = Z line; **h** and **k** = E line). **i** and **l** = *Z. mays* ssp. *mexicana* (K 69-5). The bar represents 10 µm, all with the same enlargement

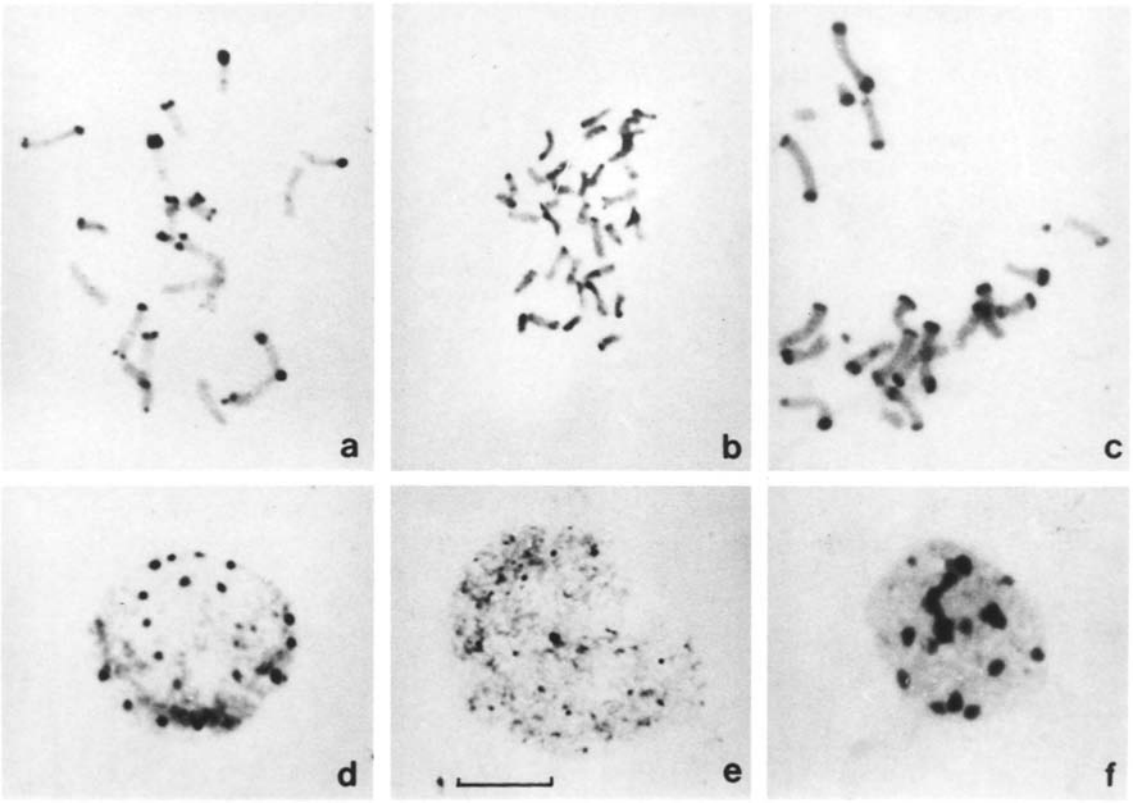

Fig. 2a–f. C-banding. **a–c** = mitotic metaphase; **d–f** = interphase nuclei. **a** and **d** = *Z. diploperennis*; **b** and **e** = *Z. perennis*; **c** and **f** = *Z. luxurians*. The bar represents 10 μm, all with the same enlargement

vegetative periods (A) and from species belonging to Section Luxuriantes. (c) *Z. luxurians* shows significant differences from the remaining taxa. (d) *Z. diploperennis* has nonsignificant differences with respect to maize lines with shorter vegetative periods (A), but differs from the rest of the studied taxa. (e) Only *Z. perennis* showed nonsignificant differences with respect to the two maize lines with lower DNA content (C-tester and "Ever green").

A positive linear relationship is shown by accessions of *Z. mays* ssp. *mays* between genome size and interval from germination to flowering (Table 1). A correspondence between the number of C+ bands in metaphase chromosomes and maximum number of chromocenters has been observed (Table 1; Figs. 1 and 2).

Discussion

Intra- and interspecific DNA content variation

The lines and populations of maize studied in the present work show a significant intraspecific variation in 2C DNA content. The lowest DNA content in the C-tester line (5.86 pg) is 15% lower than the highest one (6.87 pg) in the SC6 line. The DNA content is even higher (7.07 pg) in the E line, although in this case it would have been affected by the interaction with the cytoplasm of *Z. mays* ssp. *mexicana* (Mazoti 1987; Poggio et al. 1990b).

Laurie and Bennett (1985) and Rayburn et al. (1985) showed that 2C DNA content varies significantly within *Z. mays* ssp. *mays*, ranging from 4.9 pg in the open-pollinated line "Gaspe flint" and in the hybrid "Seneca 60" to 6.74 pg in the race "Zapalote Chico".

The DNA content of the accession of *Z. mays* ssp. *mexicana* analyzed in the present work differs significantly from the group of maize with low DNA content and shows a high 2C DNA content (6.79 pg), considering that the higher value reported by Laurie and Bennett (1985) was 2C = 6.44 pg for the accession K65-1 of *Z. mays* ssp. *mexicana*.

A hybrid between *Z. mays* ssp. *mexicana* (K69-5) and a maize with low DNA content (9063) was studied here and its DNA content was found to be intermediate between that of the parents. The difference in DNA content does not interfere with the normal pairing of chromosomes, since hybrids between *Z. mays* ssp. *mays* and *Z. mays* ssp. *mexicana* have, in general, regular meiosis showing 10 II and normal fertility (Molina and Naranjo 1987).

In the Section Luxuriantes, *Z. perennis* possesses the highest total DNA content because of its higher ploidy level, but by considering the DNA content per genome,

this species is seen to possess the lowest DNA content. This fact is not unusual, since polyploids in many groups tend to have a lower DNA content per basic genome than related diploids. This could either be due to the fact that polyploids might have been originated by diploids possessing lower DNA content or else because at the polyploid level, with duplicated genomes, the partial elimination of DNA is more easily tolerated (Martinez and Ginzo 1985; Poggio and Hunziker 1986; Bennett 1987; Grant 1987; Poggio et al. 1989; Poggio and Naranjo 1990). *Z. diploperennis*, the other perennial species in the section, has a DNA content similar to maize with low DNA content.

Two hybrids between *Z. diploperennis* and *Z. perennis* with 2n = 30 and 2n = 40 were analyzed. The origin of the hybrid with 2n = 40 was explained by the fertilization of an unreduced egg cell from *Z. diploperennis* by a normal male gamete from *Z. perennis* (Molina and Naranjo 1987; Naranjo et al. 1990). These authors postulated the following genomic constitution for *Z. diploperennis*, *Z. perennis*, and their hybrids:

Z. diploperennis (2n = 20)	× *Z. perennis* (2n = 40)
A1A1 B1B1	A1′A1′A1″A1″ C1C1 C2C2
(10 II)	(5 IV + 10 II)
Z. d. × *Z. p.* (2n = 30)	*Z. d.* × *Z. p.* (2n = 40)
A1′A1″A1 C1C2 B1	A1′A1″A1A1 C1C2 B1B1
(5 III + 5 II + 5 I)	(5 IV + 10 II)

Because *Z. diploperennis* has more DNA content per basic genome than *Z. perennis*, it is expected that the octoploid hybrid (2n = 40) between them will have more DNA content (about 6%) than *Z. perennis*. However, the observed difference between both taxa is nonsignificant because of the small expected difference, which is of a magnitude difficult to detect by the method used in the present work.

DNA content and heterochromatin

Rayburn et al. (1985) have shown that in maize the C-banding pattern correlates with the heterochromatin of pachytene knobs. Constitutive heterochromatin comprises most of the satellite DNA (Flavell 1986). Peacock et al. (1981) reported that in heterochromatic knobs of maize there is a highly repeated 185-bp satellite DNA. Rayburn et al. (1985) detected a significant positive correlation between the number of mitotic C-bands and DNA content, and between proportion of heterochromatin and DNA content. In the present work the latter relationship is seen; the C-tester line, which shows the lowest DNA content, has only four blocks of heterochromatin, while lines with more DNA (Z, E, and SC6) have ten blocks each (Table 1, Fig. 1). However, Colorado Klein variety has 12 blocks and a lower proportion of heterochromatin than lines Z, E, and SC6. This indicates that the size of bands or chromocenters is more related to DNA content than is their number.

Laurie and Bennett (1985) have pointed out that variation in the amount of heterochromatin would appear to be an important cause of differences in the DNA content of *Zea* taxa, but that it is not the only source of such variation, because taxa lacking bands do not have the lowest DNA content. Flavell (1982, 1986) pointed out that chromosomes assume the folded structure of heterochromatin when long tandem arrays of repeated sequences are clustered. If so, repeats organized in a different way (e.g., interspersed with nonrepetitive DNA and/or unrelated repetitive sequences) could not be detected by Giemsa-staining. It is interesting to note that in the Section Luxuriantes, the octoploid *Z. perennis* has the highest number of C-bands but the lowest proportion of heterochromatin (Table 1, Fig. 2). This coincides with its lowest DNA content per genome and suggests that the lost DNA consisted of repetitive sequences.

DNA content, vegetative period, and evolutionary consideration

Bennett (1972, 1987) pointed out that DNA *C*-value is positively correlated with the minimum generation time (minimum period from germination until production of the first mature seed). Rayburn et al. (1985) noted that the line with the smallest genome has a short interval from germination to flowering, while those with larger genomes have a longer one. In the taxa of maize studied in the present work there is positive correlation between genome size and interval from germination to flowering (Table 1). In the Section Luxuriantes a similar tendency is present; however, there is no positive correlation if all the taxa are considered, since *Z. diploperennis*, e.g., has a longer cycle and lower DNA content. This could be due to the fact that species in this section are dependent on photoperiod for flowering.

Price (1988a, b) stated that in cases where the phylogeny of taxa can be deduced, it is apparent that both evolutionary increases and decreases in DNA content have occurred. On the basis of the hypothesis that *Zea* has a basic number x = 5 (Molina and Naranjo 1987), the extant species are tetraploid and octoploid. Naranjo et al. (1990) have postulated that tetraploid species (2n = 20) are of alloploid origin and the octoploid *Z. perennis* is autoalloploid or alloautoploid. The ancestral diploids are extinct. For this reason any consideration about the direction of the DNA change would be entirely speculative. The extant species of the genus *Zea* would be the product of natural and artificial selection acting on different genotypic and nucleotypic constitutions at the diploid and/or the tetraploid level.

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