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Nuclear DNA content in the genera *Zea* and *Sorghum*. Intergeneric, interspecific and intraspecific variation

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Microdensitometry measurements showed that 4*C* DNA content varied significantly both within the genus *Zea* as a whole and within maize (*Zea mays* ssp. *mays*) itself. The DNA contents of diploid teosintes from Mexico and northern Guatemala (*Zea mays* ssp. *mexicana*, *Zea mays* ssp. *parviglumis* and *Zea diploperennis*) were within the range recorded for maize (9·84 to 13·49 pg), but the DNA content of a diploid teosinte from southern Guatemala (*Zea luxurians*) was about 50 per cent higher (18·29 to 18·47 pg). The DNA content of maize was three to four times greater than that of diploid *Sorghum bicolor* (3·12 to 3·47 pg). In contrast to the situation in maize no significant differences in DNA content were found between accessions of diploid *Sorghum bicolor*.

INTRODUCTION

As part of a cooperative project with CIMMYT* to investigate the feasibility of obtaining hybrids between maize (*Zea mays* (L.) ssp. *mays*) and grain sorghum (*Sorghum bicolor* (L.) Moench), the range of 4*C* DNA contents within the genera *Zea* and *Sorghum* was investigated. The rationale for these experiments was first, that the likelihood of producing karyotypically stable hybrids may be influenced by nucleotypic factors such as the relative sizes of the parental genome, and second, that a knowledge of parental genomes sizes would facilitate the unambiguous identification of genomes or individual chromosomes in any putative intergeneric hybrid.

Previously published estimates of the 4*C* DNA content for maize range from 9·4 to 25·2 pg (Bennett and Smith, 1976; Hake and Walbot, 1980; Bennett *et al.*, 1983; Barlow and Rathfelder, 1984). It is questionable whether differences of this magnitude are real, but some variation in DNA content might be expected since maize races frequently differ in the number of heterochromatic knobs (Kato, 1976; McClintock *et al.*, 1981). Unfortunately some of the previous studies did not state which maize genotype was used.

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Apart from Barlow and Rathfelder's (1984) estimate of 7·1 pg for the 2*C* DNA content of an unspecified accession of *Euchlaena mexicana* (annual teosinte), no published data are available on the DNA content of other *Zea* species or subspecies. It is therefore of interest to determine the extent of variation within and between these taxa.

The 4*C* DNA contents of several members of what is now classified as the *Sorghum bicolor* complex (De Wet, 1978), which like maize have diploid chromosome numbers of $2n = 20$, were reported to range from 11·7 to 22·8 pg (Paroda and Rees, 1971). However root-tip squash preparations show that the chromosomes of *S. bicolor* are small compared to those of maize (fig. 1) suggesting that the previously published values may have been overestimated. Several *Sorghum* taxa were examined in order to resolve this question.

MATERIALS AND METHODS

(a) Materials

Measurements of 4*C* nuclear DNA content were made on a representative collection of wild members of the genus *Zea* and on 11 stocks of maize from the United States and Mexico (table 1). Measurements were also made of eight accessions of $2n = 20$ grain sorghum (*S. bicolor* ssp. *bicolor*), 2 accessions of $2n = 40$ *Sorghum* and 1 accession of the $2n = 10$ Parasorghum *S. versicolor* (table 2).

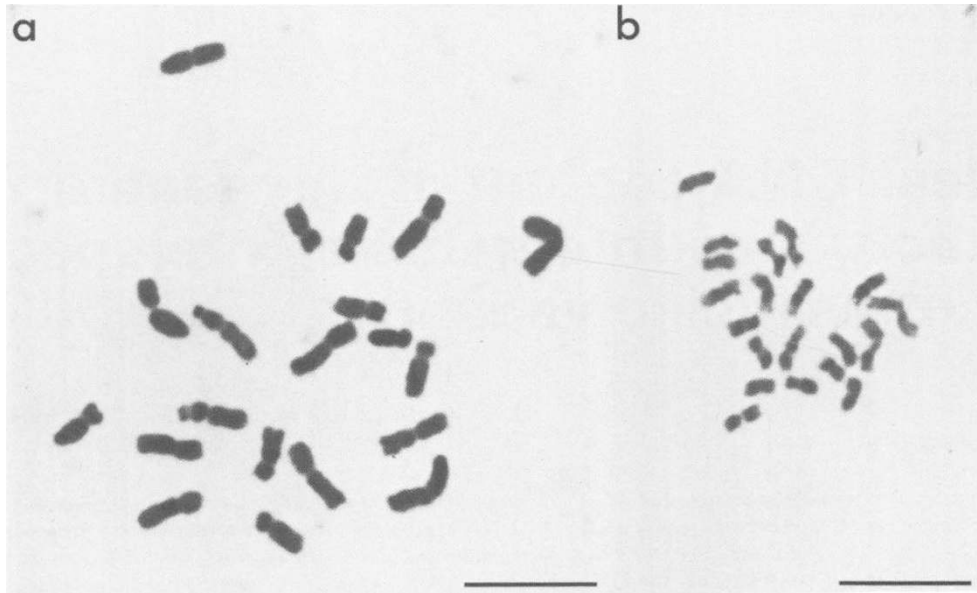

Figure 1 Root-tip metaphase chromosomes stained with Feulgen and propionic orcein. (a) *Zea mays* ssp. *mays* Seneca 60. (b) *Sorghum bicolor* ssp. *bicolor* CMS G3E A line 41-42 PR.84A. Bar represents 10 μm.

(b) Microdensitometry

Seeds were germinated on moist Whatman No. 1 filter paper in an incubator at 25°C and 3 to 4 day old root-tips were fixed for at least 24 hours in 3 : 1 ethanol/acetic acid. Fixed root-tips were rinsed in distilled water for 1 min, hydrolysed in 1N HCl for 12 min and Feulgen stained for 2 hours at room temperature. They were then given three 10 min washes in sulphur dioxide water and squashed in 45 per cent acetic acid. Three measurements of each of 10 late prophase or metaphase cells from each of three replicate slides were made on the same day using a Vickers M86 scanning microdensitometer. If less than 10 cells were found on a slide, extra slides were measured. In addition an extra slide was measured wherever possible for wild accessions in an effort to reduce possible errors arising from variation in DNA content due to polymorphism for heterochromatic segments.

Hordeum vulgare (L.) cv. Sultan (4*C* DNA content = 22·2 pg) was used as a standard for calibrating the DNA content of the *Zea* taxa and *Vigna radiata* (L.) Wilczek cv. Berken (4*C* DNA content = 2·1 pg) as a standard for calibrating the *Sorghum* taxa. The DNA content of *V. radiata* was checked against that of *H. vulgare* cv. Sultan and the value obtained (4*C* = 1·99 pg) was close to that of previous microdensitometry and reassociation kinetics experiments (Bennett *et al.*, 1982). The standard value of 2·1 pg was therefore used to calibrate all *Sorghum* taxa.

In order to ensure greater accuracy in the DNA measurements, hydrolysis curves were determined for the two standards and for *Z. mays* ssp. *mays* Seneca 60 and *S. bicolor* ssp. *bicolor* S275. In all four cases the maximum absorbance at 558 nm occurred after 12 min hydrolysis in 1N HCl at 60°C.

(c) Taxonomy

The classification of the genus *Zea* used in this paper is that given in Doebley and Iltis (1980) and Iltis and Doebley (1980), while the classification of the genus *Sorghum* is that given in De Wet (1978).

(d) Statistical analysis

Comparisons of DNA content between taxa were made using analyses of variance. In tables 1 and 5 the standard errors were calculated from the between replicate mean squares of tables 2 and 6 respectively as $\sqrt{ms/n}$ where n was the number of cells measured per accession.

RESULTS

The genus Zea

Table 1 gives the 4*C* DNA contents of 28 *Zea* accessions. An analysis of variance of the overall

Table 1 DNA content of 4*C* mitotic nuclei in the genus *Zea*

	Source	2*n*	Number of replicate experiments	4*C* DNA (pg)	SE
Section *Zea*					
Zea mays ssp. *mays*					
Commercial hybrid					
Seneca 60	1	20	4	9·84	0·202
Open pollinated variety					
Knobless Tama Flint Ac603B	1	20	1	10·28	0·367
Inbred lines					
Va35	2	20	3	10·31	0·226
Oh43	1	20	1	10·58	0·329
W64A	3	20	2	10·93	0·300
KYS	1	20	1	11·04	0·367
Races					
Palomero Toluqueño Mexico 5 BA.70 539-546	4	20	1	11·26	0·367
Chapalote Sinaloa 2 TL.72B 5-8	4	20	1	11·65	0·424
Nal-Tel Yucatan 7 TL.72B 1-4	4	20	1	11·92	0·278
Zapalote Chico Ac603A	1	20	1	13·19	0·424
Zapolote Chico Oaxaca 50 Tep.60A 5269	4	20	3	13·49	0·244
Zea mays ssp. *mexicana* (Nobogame teosinte)					
Beadle's 1974 harvest ssp. *mexicana* (Central Plateau teosinte)	5	20	1	11·01	0·382
Puga 11066	5	20	2	10·53	0·277
Doebley 625 ssp. *mexicana* (Chalco teosinte)	5	20	3	11·23	0·240
Doebley 642	5	20	1	11·85	0·377
K68-6	4	20	1	12·21	0·465
K68-1	4	20	1	12·51	0·424
K65-1	4	20	3	12·88	0·257
Zea mays ssp. *parviglumis* var. *parviglumis* (Balsas teosinte)					
Beadle and Kato Site 6	5	20	1	11·19	0·367
Puga 11065	5	20	1	11·60	0·424
Beadle's El Salado	5	20	1	11·74	0·346
K67-17	4	20	1	11·88	0·424
K67-7	4	20	1	12·39	0·439
Zea mays ssp. *parviglumis* var. *huehuetanangensis* (Huehuetenango teosinte)					
Iltis and Lind G-120	5	20	1	12·18	0·367
Section *Luxuriantes*					
Zea luxurians (Guatemala teosinte)					
Iltis G-5	5	20	1	18·29	0·424
Iltis G-42	5	20	2	18·47	0·278
Zea diploperennis (Diploid perennial teosinte)					
Iltis 1190	5	20	1	10·57	0·367
Zea perennis (Tetraploid perennial teosinte)					
Collins collection (Beadle)	5	40	1	21·13	0·367

Seed sources: (1) Professor D. B. Walden, Department of Botany, Western Ontario University, Ontario, Canada; (2) Dr J. D. Smith, Department of Soil and Crop Science, Texas A & M University, College Station, TX 77843, USA; (3) Professor J. G. Scandalios, Department of Genetics, North Carolina State University, Raleigh, NC 27695-7614, USA; (4) Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico. Collection sites for the teosinte accessions are given in Kato Y (1976) and McClintock *et al.* (1981); (5) Professor J. F. Doebley, Department of Biology, Texas A & M University, College Station, TX 77843, U.S.A. Collection sites are given in Doebley (1983) and Doebley *et al.* (1984) except for: (a) *Z. mays* ssp. *mexicana* "Doebley 642", 7·5 km SE of Chalco city limits at km 17·5 on road to Tlalmanalco 19°13′ N, 98°49′ W, altitude 2400 m; (b) *Z. mays* ssp. *parviglumis* var. *parviglumis* "Beadle and Kato Site 6", 79 km S of Valle Bravo, estimated to be 18°35′ N, 99°57′ W, altitude 900 m (Doebley pers. comm).

data revealed significant differences between species and between accessions within species despite significant variation between replicate experiments (table 2).

Variation between species was accounted for by the values for the tetraploid *Z. perennis* (21·13 pg) and the diploid southern Guatemala teosinte *Z. luxurians* (18·29 to 18·47 pg) which had 36 to 88 per cent more DNA than other diploid taxa (9·84 to 13·49 pg).

Table 1 also indicates two further levels of variation. Firstly, there was remarkable variation

Table 2 Analysis of variance of 4C DNA content in the genus *Zea*

	df	*ms*	*p*
Between species	3	2637·788	<0·025
Between subspecies within species	2	51·599	ns
Between races within subspecies	3	71·336	ns
Between accessions within species/ subspecies/races	19	58·550	<0·001
Between replicates within accessions	14	5·400	<0·001
Between slides within replicates	49	0·841	<0·001
Error	1368	0·344	
Total	1458		

The between races item compares the Nobogame, Central Plateau, Chalco, Balsas and Huehuetenango teosintes after subtracting the between subspecies variation. "Races" of maize are more properly regarded as separate accessions and all maize stocks have therefore been included in the between accessions comparison.

in maize itself which was significant despite significant differences between replicate experiments (table 3). The commercial hybrid sweetcorn "Seneca 60" had the lowest DNA content (9·84 pg) while the Mexican race Zapalote Chico (accession Oaxaca 50) had the highest (13·49), a difference of 37 per cent. Three races of Mexican maize regarded as "primitive" by taxonomists (Doebley, 1983), namely Palomero Toluqueño 11·26 pg, Chapalote 11·65 pg and Nal-Tel 11·92 pg, had DNA contents which were similar to those of teosintes from Mexico and northern Guatemala (*Z. mays* ssps. *mexicana* and *parviglumis*).

Secondly, table 1 also indicates that there may be differences in DNA content between the five "races" of teosinte classed in *Z. mays* (the Nobogame, Central Plateau and Chalco teosintes of ssp. *mexicana* and the Balsas and Huehuetenango teosintes of ssp. *parviglumis*). In particular the Chalco teosintes of ssp. *mexicana* had higher DNA contents than the Nobogame and Central Plateau teosintes of ssp. *mexicana*. The analysis in table 2 did not give a significant result for the between races comparison but this may have been because this item was tested against a between accessions item which contained all the maize stocks. Since maize itself was shown to be highly variable (see above) it may be more informative to consider these five teosintes separately. When this was done there were significant differences between races (table 4).

Table 3 Analysis of variance of 4C DNA content in maize

	df	*ms*	*p*
Between accessions	10	102·980	<0·001
Between replicates within accessions	8	3·492	<0·001
Between slides within replicates	27	0·619	<0·001
Error	643	0·280	
Total	688		

The genus Sorghum

Table 5 gives the 4C DNA contents of 11 *Sorghum* accessions. An analysis of variance of the overall data showed significant differences between species but no significant differences within species other than that due to differences in ploidy level (table 6). The difference between species was due to the value for the tetraploid *S. halepense* (6·61 pg) and to that of the $2n = 10$ Parasorghum *S. versicolor* (8·49 pg). The latter is known to have large chromosomes in comparison to those of *S. bicolor* (Gu *et al.*, 1984). The DNA contents of the diploid $2n = 20$ accessions of *S. bicolor*, the species in which all cultivated grain sorghums are classified, ranged from 3·12 to 3·47 pg but in contrast to the situation in maize this variation was not significant.

The *Sorghum* taxa examined all had lower DNA contents that those found in *Zea* (Table 1) and, not surprisingly, a between genera comparison was highly significant ($p < 0·001$).

Table 4 Analysis of variance of 4C DNA content in wild members of *Z. mays* (the Nobogame, Central Plateau and Chalco teosintes of ssp. *mexicana* and the Balsas and Huehuetenango teosintes of ssp. *parviglumis*)

	df	*ms*	*p*
Between subspecies within *Z. mays*	1	0·544	ns
Between "races" within subspecies	3	71·335	<0·025
Between accessions within taxa	8	10·257	ns
Between replicates within accessions	5	9·452	ns
Between slides within replicates	17	3·949	<0·001
Error	555	0·274	
Total	589		

As in table 2 the between races item compares the Nobogame, Central Plateau, Chalco, Balsas and Huehuetenango teosintes after subtracting the between subspecies variation.

Table 5 DNA content of 4*C* mitotic nuclei in the genus *Sorghum*

	Source	2*n*	Number of replicate experiments	4*C* DNA (pg)	SE
Section *Sorghum*					
Sorghum bicolor ssp. *bicolor*					
SII TL80B	1	20	1	3·12	0·122
S275 TL80B	1	20	1	3·20	0·122
S9B BA81	1	20	1	3·23	0·125
CMS cv. G3E A line 41-42 PR.84A	1	20	3	3·47	0·080
race caffrorum	2	20	1	3·24	0·165
race caffrorum	3	20	1	3·31	0·124
race durra	2	20	3	3·26	0·098
race nervosum	2	20	1	3·27	0·154
Sorghum bicolor ssp. *arundinaceum*					
race verticilliflorum	3	40	1	6·70	0·149
Sorghum halepense					
race almum	3	40	2	6·61	0·090
Section *Parasorghum*					
Sorghum versicolor	3	10	1	8·49	0·141

Seed sources: (1) Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), Mexico. CMS is a cytoplasmic male sterile line. (2) Zentralinstitut fur Genetik und Kulturpflanzenforschung, 4325 Gatersleben, DDR. (3) Plant Introduction Officer, Division of Plant and Seed Control, Pretoria, South Africa.

DISCUSSION

The present results reveal significant differences in 4*C* DNA content between and within taxa in the genus *Zea*. Of particular interest is the observation that maize itself shows considerable variation for this character, with the highest DNA content (13·49 pg in Zapalote Chico Oaxaca 50) being 37 per cent higher than the lowest (9·84 pg in Seneca 60). As only a limited number of accessions were studied in the present work the full range of DNA content in maize may be even greater.

Table 6 Analysis of variance of 4*C* DNA content in the genus *Sorghum*

	df	*ms*	*p*
Between species	2	571·855	<0·001
Between accessions within ploidy levels	8	0·568	ns
Between replicates within accessions	5	0·596	<0·025
Between slides within replicates	19	0·149	ns
Error	454	0·727	
Total	488		

The between species item was not tested against a between subspecies item since the latter would contain both diploid and tetraploid accessions. Instead the between species item was compared to a between accessions within ploidy levels item.

Significant variation between maize cultivars has also been found in an independent study (Rayburn *et al.*, 1985). The values obtained for the three accessions included in both investigations were in good agreement (10·28 vs. 10·19 pg for the Knobless Tama Flint, 11·04 vs. 11·20 pg for KYS and 11·92 vs. 11·22 pg for Nal-Tel).

It is of interest to consider the variation in maize DNA content in more detail. Previous workers have noted that knob number decreases with increasing latitude of cultivation for maize races grown in the U.S.A. (Anderson and Brown, 1952) and decreases with increasing altitude for maize races grown in Mexico (Bennett, 1976). Rayburn *et al.* (1985) have now shown significant positive correlations between C-band number (*i.e.*, knob number), per cent C-band heterochromatin and DNA content and have also shown that DNA content decreases significantly with increasing latitude. This provides convincing evidence that variation in DNA content in maize is largely caused by differences in the amount of heterochromatin and that previously reported correlations between geographical location and knob number involve differences in nuclear DNA content. The fact that such correlations exist suggests that these characters have adaptive significance in *Zea* and are, therefore, of potential agricultural interest.

Observations in the present study were compatible with the results cited above. For example,

Seneca 60, which had the lowest DNA content, and which was from New York State in the USA had only six blocks of heterochromatin on *C*-banded root-tip chromosomes. The Mexican race Zapalote Chico from southern Oaxaca (Oaxaca 50), which had the highest DNA content, had up to 24 C-bands including that of the K10 chromosome (fig. 2).

It seems reasonable to postulate that variation in heterochromatin also contributes to the differences in DNA content found between annual teosintes from Mexico. The present data on DNA content are consistent with data summarised in McClintock *et al.* (1981) which show that the northern races Nobogame and Central Plateau have on average fewer or smaller heterochromatic knobs than Chalco and, to a lesser extent, Balsas teosintes. However, this picture is complicated by the fact that the Balsas teosintes themselves appear to be heterogeneous (Smith *et al.*, 1982). It should be noted, however, that the teosintes appear to differ from maize in the relationship between DNA content and altitude. In contrast to the situation in maize it is the annual teosinte from the highest altitude (race Chalco) which has the highest DNA contents.

Z. luxurians (Guatemala teosinte) is conspicuously different from other $2n = 20$ members of the genus in having considerably more DNA. This provides further evidence of a clear separation of *Z. luxurians* from both maize and the remaining teosintes (cf. Timothy *et al.*, 1979; Mastenbroek *et al.*, 1981; Smith *et al.*, 1981; 1982; 1984; Doebley *et al.*, 1984).

Variation in the amount of heterochromatin would appear to be an important cause of differences in DNA content between *Zea* taxa but there are two pieces of evidence which suggest that it is not the only source of such variation. First, the Knobless Tama Flint (KTF), which is devoid of detectable C-band positive material (Mastenbroek and de Wet, 1983; Rayburn *et al.*, 1985), was found to have a significantly higher DNA content than Gaspé Flint, which has 4 C-bands (Rayburn *et al.*, 1985). Results from the present study suggest that KFT also has a higher DNA content than Seneca 60, although the KTF measurements were unreplicated. Second, *Z. luxurians* has by far the highest DNA content of the diploid *Zeas* but does not appear to have sufficient heterochromatin to account for this difference (Mastenbroek and de Wet, 1983).

The present results also show that the DNA content of *Sorghum* is much lower than previously reported and that there is no significant variation in DNA content between the $2n = 20$ *Sorghum* accessions studied. The 4*C* DNA contents of a number of $2n = 20$ *Sorghum* taxa, including three

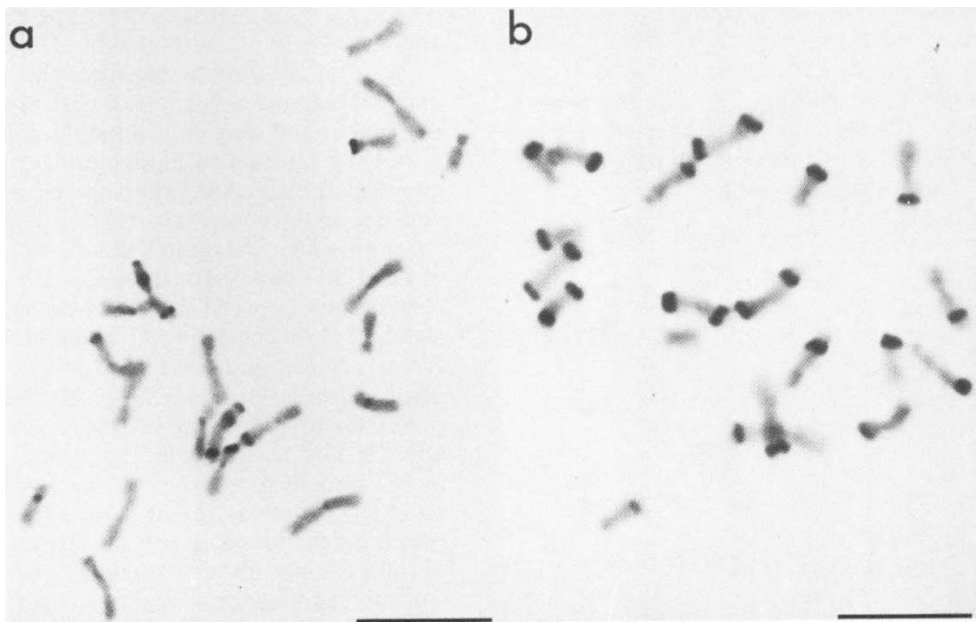

Figure 2 C-banded root-tip metaphase chromosomes. (a) *Zea mays* ssp. *mays* Seneca 60. (b) *Zea mays* ssp. *mays* Zapalote Chico Oaxaca 50. Bar represents 10 μm.

in the present study (races durra, caffrorum and nervosum), were reported to range from 11·7 to 22·8 pg (Paroda and Rees, 1971). These values are comparable to those for maize and barley (*H. vulgare*) respectively (Bennett and Smith, 1976; Bennett *et al.*, 1983; this paper), but measurements made in this laboratory estimate the 4*C* DNA content to be in the range 3·0 to 3·47 pg for $2n = 20$ accessions (Bennett *et al.*, 1983 and this paper). The reason for this discrepancy may lie in the choice of cells measured. Paroda and Rees (1971) measured interphase nuclei and "... mean 2*C* values were obtained from the 10 lowest readings among the 20 nuclei presumed to be 2*C*". In the present study only 4*C* late prophase cells were used but large interphase nuclei containing up to 10 pg of DNA were found. Perhaps the previous estimates were unduly high because of the inclusion of polyploid cells.

A comparison of the DNA contents in tables 1 and 5 shows that the smallest estimate for maize (Seneca 60) is 2·8 fold larger than the largest estimate for *S. bicolor* (CMS), while the largest estimate for maize (Zapalote Chico Oaxaca 50) is 3·9 fold larger than the smallest estimate for *S. bicolor* (SII). This difference suggests that there may be difficulties in hybridising these genera since work on other crops indicates that stable hybrids are not usually produced in situations where parental genomes with equal chromosome numbers differ so greatly in size. Nonetheless if hybrids are produced the differences in genome sizes between the parents should be sufficient to enable the parental origin of each individual chromosome to be identified with certainty.

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